Quantitative genetics of *Eucalyptus globulus*, *E. nitens* and their F_1 hybrid

by

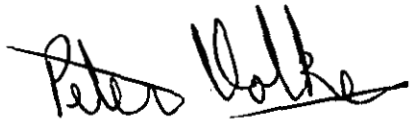
Peter William Volker

BSc (Forestry) GradDipSc *ANU*

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, University of Tasmania (April, 2002)

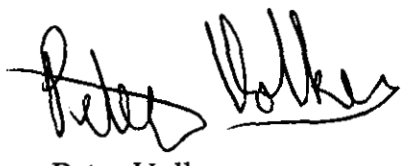
Declarations

This Thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by any other person except where due acknowledgment is made in the text of the Thesis.



Peter Volker

This thesis may be made available for loan and limited copying in accordance with the Copyright Act 1968.



Peter Volker

Abstract

This thesis examines the quantitative genetics of intra- and inter-specific hybrids of *E. globulus* ssp. *globulus* and *E. nitens*. The trials established to make this study are unique in forestry, due to the fact that the same parents have been used in open-pollination, intra- and inter-provenance (or intra-species) crosses and inter-species F_1 hybrids. This has allowed direct comparison of genetic parameters derived from different cross types. The traits examined include frost resistance using an electrical conductivity method, growth (diameter at breast height over bark DBHOB, at ages 2, 3, 4, 6 and 10 years) and Pilodyn penetration at age 6 years as an indirect measure of wood density.

The results demonstrate that the measured performance of frost, growth and Pilodyn traits in the inter-specific F_1 hybrid *E. nitens x globulus* is always intermediate or comparable with one or other of the parent species. In the frost trait, the inter-specific hybrid was no better than the frost sensitive *E. globulus*, so there is no overall advantage in producing the hybrid for this trait. Negative mid-parent heterosis was observed for early age growth traits in inter-specific *E. nitens x globulus* F_1 hybrids involving Taranna *E. globulus* male parents. The inter-specific F_1 hybrids demonstrated generally poor survival and a high proportion of abnormal and slow growing phenotypes, which eventually died. This latter phenomenon was not evident in pure species crosses, either within or between provenances.

It is shown that in *E. globulus* open-pollinated progeny estimates of additive genetic parameters are inflated and that breeding values for growth in *E. globulus* are poorly estimated, possibly due to the confounding effects of variation in inbreeding. This was not the case for traits of high heritability such as Pilodyn. Within the *E. nitens* population studied, open-pollinated estimates compared well with control-pollinated estimates for all traits.

Genetic parameter estimates from control-pollinated progeny indicate low heritability for growth in *E. globulus* which diminish over time. Dominance effects were low and comparable with additive genetic effects but were site specific. In *E. nitens* heritability for growth is moderate to high, tending to increase over time with significantly low levels of dominance, which diminish over time. Pilodyn has low to moderate heritabilities with low levels of dominance in both species. Moderate levels of heritability were demonstrated for frost resistance in both species, but dominance effects could not be accurately estimated.

The correlation of performance of parents in intra-specific crosses through their General Combining Ability (GCA) is compared with performance in inter-specific hybrids through General Hybridising Ability (GHA). It is shown that there is little or no correlation between GCA and GHA in inter-specific F_1 hybrids for growth or frost resistance, but there was a good correlation for Pilodyn. This indicates that, for growth and frost resistance, there may be different genes, which contribute to expression between species and these may not combine according to classical quantitative genetic theory. In contrast, within *E. globulus* there was very high correlation of within-provenance GCA with between-provenance GHA for growth and Pilodyn, indicating the same genes are acting within the species, regardless of provenance.

It is demonstrated that standard quantitative genetic models do not cope adequately with inter-specific F_1 hybrid populations for growth traits in this case. In addition, the implication for breeding and deployment of inter-specific F_1 hybrids is compromised by the lack of ability to predict performance of potential hybrid combinations from pure species performance of parents.

Publications arising from this project

Gore, P.L., Potts, B.M., **Volker, P.W.** and Megalos, J. (1990) Unilateral cross-incompatibility in *Eucalyptus*: the case of hybridisation between *E. globulus* and *E. nitens*. *Australian Journal of Botany* 38, 383-94.

Potts B.M., **Volker P.W.** and Dungey H.S. (1992). Barriers to the production of interspecific hybrids in *Eucalyptus*. *In* 'Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species.' Proc. IUFRO/AFOCEL Symposium, Bordeaux, France, Sep. 1992 pp. 193-204. (AFOCEL, Paris).

Dungey, H., **Volker, P.W.**, Potts, B.M. and Owen, J. (1993) Inter-specific hybridisation between *Eucalyptus nitens* and *E. globulus*: preliminary analysis of early growth and frost resistance. In Proceedings of Meeting of Research Working Group One (Forest Genetics) of the Australian Forestry Council held in Canberra, ACT 15-18 Feb. 1995 (ed. M.U. Slee). (Australian National University, Canberra).

Volker P.W., Borralho N.M.G. and Owen J.V. (1994). Genetic variances and covariances for frost tolerance in *Eucalyptus globulus* and *E. nitens*. *Silvae Genetica* **43**, 366-372.

Volker, P.W. (1995). Evaluation of *Eucalyptus nitens x globulus* for commercial forestry. In 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Conference, Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 222-225. (CRC for Temperate Hardwood Forestry, Hobart).

Potts, B.M., **Volker, P.W.**, Hodge, G.R., Borralho, N.M.G., Hardner, C.H. and Owen, J.V. (1995). Genetic limitations to the exploitation of base populations of *Eucalyptus globulus* ssp. *globulus*. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Conference, Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 217-221. (CRC for Temperate Hardwood Forestry, Hobart).

Vaillancourt, R.E., Potts, B.M., Watson, M., **Volker, P.W.**, Hodge, G.R., Reid, J.B. and West, A.K. (1995). Detection and prediction of heterosis in *Eucalyptus globulus*. *Forest Genetics* **2**, 11-19.

Hodge G.R., **Volker P.W.**, Owen J.V. and Potts B.M. (1996). A comparison of genetic information from open-pollinated and control-pollinated progeny tests in two eucalypt species. *Theoretical and Applied Genetics* **92**, 53-63.

Potts, B.M., **Volker, P.W.**, Tilyard, P.A. and Joyce, K. (2000). The genetics of hybridisation in the temperate *Eucalyptus*. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium, 9-14 April 2000, Noosa, Queensland, Australia. (Eds. Dungey, H.S., Dieters, M.J. and Nikles, D.G.) pp. 200-211. (Queensland Department of Primary Industries, Brisbane)

Acknowledgments

The patience and guidance of my supervisor, Brad Potts, has been invaluable in achieving an outcome to this marathon project. I also thank associate supervisor, Nuno Borralho, for his assistance, enthusiasm and encouragement, especially during his term at the CRC for Temperate Hardwood Forestry.

The project was commenced in 1986 by CSIRO Division of Forestry and Forest Products with funding provided by the Australian Special Rural Research Fund and North Forest Products (now Gunns Ltd.) under the leadership and guidance of Dr. Rod Griffin. During this time at Ridgley, Ian Whyte, David de Little and Ian Ravenwood of North Forest Products provided valuable support to the project and staff.

I thank Peter Gore, Kim Badcock and Milton Savva who worked with me as technical officers with CSIRO at Ridgley between 1987 and 1990. As a team we undertook an ambitious control-pollination program and subsequent trial establishment on a number of sites throughout Australia. John Owen (CSIRO, Canberra) was instrumental in getting the trials established and maintained after I left CSIRO.

Data has been collected over the years by staff from CSIRO, the CRC for Temperate Hardwood Forestry and CRC for Sustainable Production Forestry as well as industry partners where trials were located (Gunns Ltd – formerly NFP, Forestry Tasmania, WAPRES – formerly Bunnings Tree Farms, Grand Ridge Plantations - formerly AP Plantations, Norske Skog – formerly ANM Forest Management).

There are many colleagues in the forest genetics community who have encouraged, assisted and provided guidance during this project including Ken

Eldridge, Paul Cotterill, Christine Dean, the late Prof. Lindsay Pryor, Sandra Hetherington, Tim White, Gary Hodge, Carolyn Raymond, Jim Reid, Heidi Dungey, Greg Dutkowski, Greg Jordan, Bertrand Cauvin, Jean-Noel Marien, Peter Kanowski, Phil Whiteman, David Boomsma, Luis Apiolaza and Gustavo Lopez.

I am forever indebted to Kereth, Meaghan and Sam for being so patient during the decade in which this thesis took to produce. I also thank Moira and Jan Volker for providing me with the life skills, education and opportunities to realise my dreams.

Table of Contents

Chapter 1: Introduction

Tree breeding history

The breeding of forest trees is a relatively new phenomenon compared to the long history of crop breeding. Specific varieties of trees have been developed for ornamental use for hundreds of years, however until the widespread use of trees in intensively managed plantations on very large areas in the 20th Century there was probably little perceived need for active tree breeding programs. Breeding programs for commercial forest species were relatively few until the 1930's when activity appeared to be starting in earnest (Wright 1976). Many of these programs were interrupted during the Second World War and resumed in the 1950's. The main activity was in *Pseudotsuga menziessi* in NW USA and Europe, southern pines in SE USA and Queensland, Australia, *Pinus radiata* in Australia and New Zealand, *Pinus sylvestris* in Britain and Europe, *Picea* species in Scandinavia, *Populus* in Europe, USA and Australia (Zobel and Talbert 1984).

In most cases government institutions or Universities undertook these programs, as long lead times involved with testing and selection made them unattractive for industrial investment. Industry generally participated through funding research in these institutions, buying the products of the breeding program as seed and participating in co-operative breeding programs with other companies providing funds for the work to be carried on by the institutions.

Private investment in tree breeding programs is now common for the following reasons:

- large scale plantation developments over many thousands of hectares often owned and operated by a single industrial processor (Brown 2000);
- improvements in silviculture (Cromer *et al.* 1975; Campinhos 1980; Talbert 1995);
- demonstrated genetic advancement in growth and other traits (Shelbourne 1991; Perrow (MacRae) and Cotterill 2000; Wei and Borralho 2000);
- increased understanding of relationship between fibre properties and production processes (Bamber 1985; Malan 1988; Bouvet and Bailleres 1995; Dean 1995; Fonseca *et al.* 1995; Raymond 1995; Tibbits *et al.* 1995; Williams *et al.* 1995; Zobel and Jett 1995; Greaves and Borralho 1996; Greaves *et al.* 1997a; Kibblewhite *et al.* 1998; Cotterill *et al.* 1999);
- documented breeding strategies which have led to reduction in generation times through greater focus on early selection (Cotterill 1984; Cotterill 1986a; Cotterill 1986b; White 1987);

- the development of vegetative propagation techniques, enabling clonal forestry (Campinhos and Ikemori 1984; Wilson 1993; Zobel 1993; Bertolucci *et al.* 1995; Wilson 1995; Borralho 1997; Cotterill and Brindbergs 1997; MacRae and Cotterill 1997; Haines 2000);

- international trade in seed, selected propagules and genetic information.

The scale and complexity of some programs has led to the establishment of national (Arnold *et al.* 1991; Greaves 1994; Jarvis *et al.* 1995) and international co-operative breeding programs (Davidson 1977).

Eucalypt breeding

Eucalyptus had become the most widely planted hardwood genus in the world by the early 1990's and the annual plantation establishment has continued to expand (Brown 2000). While eucalypts were being planted in many places throughout the tropics and warm temperate areas of the world there was very little formal tree breeding activity until the 1980's (Eldridge *et al.* 1993). The earliest recorded evidence of formal studies of crossing and breeding activity comes from the Balkans in Russia by Pilipenka (1969) who reported on studies of eucalypt hybrids made in the 1930's. There were other studies of eucalypt genetics and genecology which have been reviewed by Eldridge *et al.* (1993) and Potts and Wiltshire (1997). These reviews show that most research studied patterns of variation in natural stands, species trials or provenance tests and tried to explain spatial variation in morphological differences.

In recent times, there has been much international activity in breeding and selection of *Eucalyptus*. There have been a number of international conferences with genetic improvement of eucalypts as a major theme in the last decade (Schonau 1991; Potts *et al.* 1995a; Higa *et al.* 1997; Dungey *et al.* 2000b; Anon. 2001). Examples of more advanced breeding programs include: *E. grandis* and various other species in South Africa (van Wyk 1985; van Wyk 1990; Verryn 2000), Florida, USA (Franklin 1986), *E. grandis* hybrids in Brazil (Brune and Zobel 1981; Campinhos and Ikemori 1989), *E. robusta* in Florida (Dvorak *et al.* 1981), *E. gunnii* and hybrids in France (Cauvin 1983; Potts and Potts 1986), *Eucalyptus urophylla x grandis* in Congo (Chaperon 1977; Cremiere 1988; Vigneron 1988; Vigneron 1995), *E. globulus* in Portugal (Borralho 1992; Almeida *et al.* 1995), Spain (Soria and Borralho 1998), Chile (Arnold *et al.* 1991; Jayawickrama *et al.* 1993), Argentina (Lopez *et al.* 2001b) and Australia (Jarvis *et al.* 1995) and *E. nitens* in Australia (de Little *et al.* 1993; Tibbits and Hodge 1995), Chile (Jayawickrama *et al.* 1993) and New Zealand (Cannon and Shelbourne 1991; Gea *et al.* 1997).

Basic quantitative genetic parameters, such as the proportion of additive genetic variance (expressed as narrow sense heritability) and genetic correlation between traits for eucalypts were not reported until the 1970's (Eldridge 1971; Eldridge 1972; van Wyk 1976; 1977; Kedharnath and Vakshasya 1978). There was very little additional reporting until the early 1990's indicating that very few formal studies of breeding had been undertaken (Eldridge *et al.* 1993 p.175; Potts and Wiltshire 1997). *Eucalyptus globulus* is the species in which probably the most reports of narrow sense heritabilities of growth traits and wood quality have been made (Lopez *et al.* 2001a). Advanced generation breeding and selection on a large scale with *Eucalyptus* is a relatively recent phenomenon. Despite this lack of breeding work, eucalypts have become the most widespread genera used in plantation

forestry throughout the world (Brown 2000). Seed has been obtained from collections in:

- natural stands in Australia;
- plantations;
- individual trees or groups of trees in parks and gardens;
- special plantings such as seedling seed orchards which were usually derived from phenotypic selections of better performing trees in plantations (Eldridge *et al.* 1993).

In the first case there was little or no knowledge of genetic variation and other relevant parameters. Provenances were separated on the basis of geographic characteristics rather than genetics. In the latter three cases the genetic background of the selections was usually indeterminate. It is thought that where land races (defined by Zobel and Talbert 1984 p.89; and further refined by Eldridge *et al.* 1993) have developed from these original seed collection activities, there has been a build up of inbreeding and on-average, poorer performance with each generation (Eldridge *et al.* 1993). It is only where land races have been based on a large number of unrelated individuals that there has been an opportunity for ongoing genetic improvement. Many organisations involved in eucalypt plantations have commenced broad-based breeding programs, often returning to native eucalypt stands to broaden the genetic base (Eldridge 1995).

Tree breeding programs rely on the exploitation of additive genetic variance or general combining ability (GCA) (Zobel and Talbert 1984; Eldridge *et al.* 1993). It is only where there is an opportunity for mass controlled pollination

or vegetative propagation that dominance genetic variance or specific combining ability (SCA) can be exploited (Zobel and Talbert 1984; Shelbourne *et al.* 1997; Rezende and de Resende 2000), generally through the use of reciprocal recurrent selection (Allard 1960; Matheson 1990; Bouvet *et al.* 1992; Dieters and Dungey 2000; Kerr *et al.* 2000; Potts and Dungey 2001).

Despite the aforementioned early lack of genetic studies in eucalypts, breeding and selection programs proceeded via seed production stands and open-pollinated seed orchards (Eldridge *et al.* 1993). Inter-specific hybrids have been selected for commercial vegetative propagation since the early 1960's (Franclet and Boulay 1982; Campinhos and Ikemori 1984; Martin 1989; Campinhos 1999; Dungey and Nikles 2000). In this time the use of hybrids has become widespread, despite the uncertainty of the genetic background of the material used (Eldridge *et al.* 1993; Dungey and Nikles 2000).

Most early stages of domestication involve exploitation of base populations derived from range wide open-pollinated seed collections. The efficiency of selection from such populations depends upon the accuracy of genetic parameters derived from open-pollinated progeny. The present study explores the genetic variation within and between two provenances of *Eucalyptus globulus.* A direct comparison of genetic parameter estimation through use of open-pollinated and control-pollinated progeny from the same parent is examined for *E. globulus* and *E. nitens.* This approach has been used to further explore the confounding effects of inbreeding depression and varying levels of outcrossing among individuals derived from native stand populations on genetic parameter estimation in *Eucalyptus* (Chapter 4).

Use of hybrids in forestry

There has been widespread use of hybrids in commercial plantations. The most widespread use of hybrids probably occurs with commercial plantations of poplars (*Populus* spp.) for pulp and timber production on all continents. Hybrid production has also been practiced with softwood genera such as *Picea* and *Pinus*, and hardwood genera such as *Betula*, *Eucalyptus* and *Quercus* (Dungey 2000; Dungey and Nikles 2001). In most cases the hybrids have been used in a particular environmental niche where they performed better than local species or are in an exotic environment. In the latter case the hybrid introduction has often been preceded by the introduction of one or both of the parental species; *e.g. Eucalyptus grandis, E. urophylla* in Brazil (Campinhos and Ikemori 1989) and *Pinus elliottii, P. caribbea, P. hondurensis* in Queensland (Nikles and Robinson 1989). The hybrids have often demonstrated an advantage over the parental species, generally resulting in higher production. The key to unlocking the productive potential of hybrids has either been by the use of mass controlled pollination systems that produce large amounts of hybrid seed or through vegetative propagation.

A weakness with many of these programs, until recently, is that once a successful hybrid has been discovered and propagated successfully for commercial programs, the breeding programs have lapsed until a problem arises with the clones in use (Dungey 2001). This situation became evident in *Populus* programs around the world, where only a few commercial clones were in use on a world-wide basis. The onset of poplar rust, caused by *Melampsora larici-populina* and *M. medusae*, had a significant negative impact on growth of popular commercial clones and led to renewed breeding efforts to find resistant clones (Willing and Pryor 1976).

An example of an advanced hybrid breeding program is the sub-tropical *Pinus* breeding strategy in Queensland, Australia (Nikles 2000). This program uses the *P. caribbea, P. hondurensis, P. elliotii* and *P. tecumanii* in various combinations. The program has advanced to F_3 for *P. caribbea x hondurensis* and F_2 seed has been produced in seed orchards for commercial plantations (Nikles 2000). Nikles (1992) has proposed reciprocal recurrent selection involving separate populations of parental species as a strategy to maintain continued improvement in the hybrid.

The production of hybrids has generally been pursued with the aim of achieving complementarity of traits (Martin 1989). In forestry, most of the hybrids in use involve crosses between two geographically separated species (Dungey 2001). The aim of these crosses has been to overcome disease problems, which may be present in one of the parental species (Campinhos and Ikemori 1989), or to develop a tree that is suited to a particular environment perhaps unsuited to either parental species (Martin 1989).

Pryor (1961) suggested that hybridization would be a means of improving eucalypts for wood production. He suggested that F_1 hybrids offered the opportunity for securing, in combination, sets of characters derived from two parent species, some of which may resemble those of one or other parent, and others which are intermediate between the two parents.

Requirements for complementarity also occur where species that are more easily propagated by vegetative means are hybridized with those that are difficult to propagate but may contain some other favourable production traits. Another case is where frost resistant species are combined with susceptible species that have better growth, form or wood quality characteristics (Comer *et al.* 1983; Potts *et al.* 1986; Meskimen *et al.* 1987; Cauvin 1988; Tibbits *et al.* 1989; Rockwood 1991; Tibbits *et al.* 1991b).

This idea of complementarity has been pursued recently with combinations of *E. globulus*, which is a premier wood for Kraft pulp production, in combination with *E. grandis* or *E. camaldulensis* for propagation ability and other traits (Dale *et al.* 2000; Griffin *et al.* 2000; McComb *et al.* 2000; Myburg *et al.* 2000) and *E. nitens* or *E. gunnii* for frost tolerance (Potts *et al.* 1987; Tibbits *et al.* 1989; Tibbits *et al.* 1995).

Complementarity vs hybrid vigour

The concept of hybrid vigour and heterosis are often used interchangeably in forestry. The importance of heterosis is a key issue in the exploitation of hybrids in forestry (Nikles and Griffin 1992). There are early reports of hybrid vigour in inter-specific F_1 hybrids of *Eucalyptus* (e.g. Venkatesh and Sharma 1977a). However, intra-specific controls are often absent or of poor accuracy (i.e. open-pollinated or unrelated to the F_1) making it difficult to assess whether differences between hybrid and pure material has been achieved simply through removing inbreeding effects by wide intra-specific outcrossing (Eldridge *et al.* 1993).

If hybrid superiority is shown to be the result of true genetic heterosis, (dependent upon dominance effects - Allard 1960) this would open the possibility of exploiting it by crossing highly differentiated populations (Nikles 1992). Unfortunately, this approach may be countered by the potential for outbreeding depression arising from incompatibilities between markedly divergent genomes (Orr 1995), the potential increasing with increasing taxonomic distance between parents (Potts *et al.* 1987; Griffin *et al.* 1988; Ellis *et al.* 1991; Baril *et al.* 1997a; 1997b). Not surprisingly, there have been conflicting results when relating genetic distances with the presence of heterosis.

Hybrid superiority in forest tree genera is unlikely to result from heterosis *per se* (Ledig 1986; Nikles 1992; Nikles and Griffin 1992), but more likely from the

beneficial combination of independent complementary traits (each under predominantly additive genetic control) (Ledig 1986; Martin 1989; Nikles 1992; Nikles and Griffin 1992). In the latter case, hybridization of species with complementary traits may result in synergistic effects in specific environments where neither species is well adapted (Martin 1989; Sedgley and Griffin 1989).

In addition hybrids have also been successful where they have involved crosses between land races of species in foreign countries. Such is the case with eucalypts outside Australia. It is suspected that so-called hybrid vigour is really a manifestation of a release from inbreeding rather than a heterotic effect (Eldridge *et al.* 1993).

Genetic prediction of hybrid performance

Prediction of F_1 hybrid performance is important to development and selection of material for commercial tree breeding programs, as most efforts to breed hybrids have focussed on the F_1 hybrid generation. Such prediction will depend upon:

(i) the importance of specific combining ability, relative to general combining ability (SCA vs GCA), i.e. the relationship between dominance and additive genetic effects, at the species, provenance and individual level; and

(ii) the comparability of genetic parameters estimated from hybrid and pure species populations (i.e. GCA vs GHA), explained below.

Nikles and Newton (1991) raise the concept of General Hybridising Ability (GHA) as a measure of the additive performance of a trait in hybrid combination. Attempts have been made to determine whether General Combining Ability (GCA) is a good predictor of GHA in tropical pine hybrids

between *Pinus caribbea* and *P. elliottii* (Nikles and Newton 1991; Powell and Nikles 1996; Gwaze *et al.* 2000). There is little published information where comparisons between hybrid and pure species, i.e. GHA vs. GCA, have been based on the same parents in eucalypts (Potts and Dungey 2001).

Development of eucalypt hybrids

The great majority of traditional eucalypt breeding has focussed on improvement of pure species (Eldridge *et al.* 1993). In many cases this has involved bringing together selected trees from provenance trials of species with widespread and often disjunct populations (Eldridge *et al.* 1993). In these cases it has been shown that genetic advances in these intra-specific "hybrids" may partly be attributable to release from inbreeding depression which is evident in natural populations (Hardner *et al.* 1998).

Brown (1972) considered that inter-specific hybridization of eucalypts would remain a technique of minor significance in tree breeding until the problems of reproduction could be solved. This was achieved in the early 1980's to the extent that Griffin *et al.* (2000) state, *"It has become clear the greatest advance in industrial plantation forestry of the past 20 years has undoubtedly been the clonal deployment of hybrid genotypes. These hybrid genotypes are simply good phenotypes, which have satisfied the criteria of a widespread screening program for coppicing, vegetative propagation, growth, and wood properties."* The superior growth performance is often a result of environmental adaptations of the hybrids compared with either of the parental species (Griffin *et al.* 1988; Campinhos and Ikemori 1989; Martin 1989). The most famous examples are the *E. urophylla x grandis* (*E. urograndis*) clones selected from spontaneous hybrids in the Congo (Vigneron and Bouvet 2000) and Brazil (Campinhos and Ikemori 1984). In particular, hybrids of the sub-tropical eucalypts including various combinations of *E. grandis, E. urophylla, E. tereticornis* and *E. camaldulensis* have

been used in forestry for many years in India, Brazil (Campinhos and Ikemori 1989), Congo (Martin 1989), Morocco and South Africa through use of seed or cuttings (Eldridge *et al.* 1993). While many early selections were often from sporadic eucalypt hybrids occurring in seed collections derived from native stands or exotic species trials (Bowden 1964; Vigneron 1991; Eldridge *et al.* 1993), there is now increasing interest in specifically breeding hybrid eucalypts for deployment (Venkatesh 1982; Martin 1989; Vigneron 1991; Denison and Kietzka 1992; Endo and Lambeth 1992; Nikles and Griffin 1992; Bertolucci *et al.* 1995; 1996b; Bouvet and Vigneron 1996c; Liu *et al.* 1996; Wright 1997; Shelbourne 2000; Verryn 2000).

Despite the success of these hybrid propagation programs very little genetic improvement has been achieved (Griffin *et al.* 2000). Given the unimproved nature of the parental species and the serendipitous origin of many of the current generation of industrial clones, it is unreasonable to assume that a plateau with respect to improvement of yield and quality has been reached.

Griffin *et al.* (2000) summarizes the reasons that have limited *Eucalyptus* breeders from producing new superior candidate clones as:

a) starting with the wrong parental genotypes;

b) not producing and testing large enough hybrid populations to permit adequate selection pressures;

c) seeking operational selections in the F_1 generation when it is more likely that optimal genotypes will lie in more complex advanced generation and backcross combinations.

Breeding strategies for genetic improvement of *Eucalyptus* hybrids have been suggested (de Assis 2000; Perrow (MacRae) and Cotterill 2000; Shelbourne 2000; Vigneron and Bouvet 2000). In all cases, the strategies recognise the

problem of predicting hybrid performance using various types of base populations ranging from original native populations to hybrid populations of unknown genetic origin.

Shelbourne (2000) states, *"Further development of successful hybrid breeding and deployment depends on a number of overlapping factors, including:*

- *crossability of the two species;*
- *feasibility of controlled pollination;*
- *viability of hybrid offspring;*
- *vegetative propagability* (sic) *of mature hybrids, or of juvenile hybrid material;*
- *feasibility of F_1, F_2 or backcross hybrid seed production;*
- *costs of breeding and deploying hybrids;*
- *gains in different traits from hybrids, versus from improvement of pure species."*

The use of *Eucalyptus* hybrids to date has resulted from phenotypic selection of individuals that are propagated by vegetative means. This is followed by a period of performance evaluation of propagules before a particular clone is produced on a commercial scale. Knowledge of genetic parameters has the potential to reduce the time taken for these steps and to return more information to the tree breeder to improve accuracy of selection for propagation.

The case of *Eucalyptus nitens x globulus*

E. globulus is recognised as the premier eucalypt for the Kraft pulping process. Where possible, *E. globulus* is preferred because of its high pulp yield and

wood density, which are both strongly correlated to wood costs in chemical pulping processes (Greaves *et al.* 1997a). The natural *E. globulus* distribution is limited by minimum temperature in Australia (Jordan *et al.* 1994; Dutkowski and Potts 1999) and it is also confined to relatively frost free plantation environments in temperate zones of Australia, Chile, Spain, Portugal, Italy, California and China (Eldridge *et al.* 1993).

E. nitens is seen as an ideal replacement for *E. globulus* in plantations as it has been demonstrated to be frost tolerant (Volker *et al.* 1994; Tibbits and Hodge 2001). It shows a featured grain in timber or veneer because of distinct differences between earlywood and latewood, whereas *E. globulus* tends to have a more uniform colour and texture. The pulping features of *E. nitens* have been evaluated for the Kraft process (Tibbits and Hodge 1995; Beadle *et al.* 1996; Tibbits and Hodge 1998) and are quite promising but for different paper sectors than *E. globulus* (Cotterill and Brolin 1997). This leads to different markets and uses due to preference and utility.

These two species therefore, cover a range of complementary characteristics, making the development of a hybrid between them useful for industrial purposes in temperate plantation zones (Tibbits *et al.* 1995). In addition there are a number of other eucalypts that may be suitable for hybridization to extend the range of "*globulus* like wood" in cool temperate climatic zones or arid zones of the world.

There are few investigations of hybridization reported in the literature, where the same parents were used in pure species and hybrid crosses in forest trees (Nikles and Newton 1991; Dungey 2001). In many studies of hybridisation in eucalypts, the case for heterosis has been made at the species level but failed to examine the genetic performance of individual parents in each species (1977b; Venkatesh and Sharma 1977a; Paramathma *et al.* 1997).

Project Aim

The aim of this project is to examine the genetic parameters and performance of *Eucalyptus* parents and progeny resulting from open-pollination, within species cross-pollination (within and between populations), and between species cross-pollination. A study of this type and scale has never been attempted before in forest trees including *Eucalyptus*. In this case *E. globulus* ssp. *globulus* (hereafter referred to as *E. globulus*) and *E. nitens* have been used.

In the case of inter-specific hybrids the study addresses, in particular, three of the issues raised by Shelbourne (2000). These are:

- crossability of the two species
- viability of hybrid offspring
- gains in different traits from hybrids, versus from improvement of pure species.

This study describes results of intra- and inter-specific hybridization in *E. nitens* and *E. globulus* for a number of traits including survival (Chapter 2), frost resistance (Chapter 3), growth at various ages and Pilodyn penetration (Chapters 4 and 5). The main emphasis is on the correlation of GCA and GHA, *i.e.* do the best parents within a provenance or species produce the best intra- or inter-specific hybrids respectively (Chapter 5). The design of the trial is unique in studies of eucalypt hybrids to date, in that the same parents have been used in hybrid crosses as have been used in pure species crosses. The mating design allowed estimates of additive and non-additive genetic variance to be made for intra- and inter-specific crosses.

Open-pollinated seed was also collected from the parents used in the control-pollination program of both *E. nitens* and *E. globulus*. This enables direct

comparison of genetic parameters derived from open-pollination, where only the female parent is known and control-pollination, where both parents are known, therefore making the estimates more reliable (Chapter 4).

Due to limitations in the amount of seed that could be produced, inter-species hybrids were planted on three sites, with one site, where the complete set of available material was planted, used for the main analysis (Chapter 5). Intra-species crosses within *E. globulus* and open-pollinated families from *E. globulus* and *E. nitens* were planted on multiple sites in southern Australia (described in Chapter 2).

Chapter 2: Crossing Program, Seed Yields, Trial Design and Establishment

Introduction

This chapter describes the crossing program and field trial designs used to achieve the aims of the project.

Sensitivity to low temperature is a major factor limiting the expansion of eucalypt plantations in Tasmania. A consequence of this has been the use of the cold tolerant *E. nitens* (Tibbits and Reid 1987a; 1987b) in increasing proportions of Tasmanian plantations since the early 1980's (Tibbits 1986). Expansion of the area of *E. nitens* plantations has generally been at the expense of the cold-sensitive *E. globulus* for which land was not readily available. Other species that have had plantation establishment reduced since *E. nitens* came into use are *E. regnans, E. obliqua* and *E. delegatensis*.

A hybrid combination of *E. globulus* and *E. nitens* is attractive to the pulp and paper industry in Tasmania to combine the following traits from each species.

E. globulus: high chemical pulp yield, branch shedding ability, coppicing ability and resistance to *Chrysoptharta bimaculata* defoliation

E. nitens: frost resistance, high volume growth, wood quality which is comparable to the highly desired *E. regnans* for solid timber, veneers and chemi-mechanical pulping processes.

At present, plantations of *E. nitens* are susceptible to widespread defoliation by the insect *Chrysoptharta bimaculata*, a problem which can only be treated by

application of insecticide and long-term breeding for resistance. Also, retention of dead branches on stems increases susceptibility to heart rot in the wood.

A hybrid that combines the traits described above would be utilised in the intermediate plantation zones between the coastal region (below about 300m) and the high altitude plateau regions (above 600m). In this zone winter frosts are common, with minimum temperatures between 0 and -5°C and extremes to -13°C. Where frosts are not a problem, then *E. globulus,* is preferred for plantation establishment. The widespread use of the *E. nitens x globulus* hybrid is also dependent on the ability to produce sufficient quantities of seed or vegetative propagules by micro-propagation or cuttings.

Project Objectives

The project objectives are dealt with in detail in each chapter as follows:

a) Examine the feasibility of producing *E. nitens x globulus* hybrid material for use in operational breeding and deployment programs (Chapter 2 and 5).

b) Determine the extent to which General Combining Ability (GCA) of *E. globulus* and *E. nitens* in intra-specific combination is predictive of performance as parents in hybrid combination, *i.e.* General Hybridising Ability (GHA) (Chapter 5).

- Are GCA estimates from intra- and inter-provenance crosses within a species comparable? (Chapters 3 and 4)

- Does heterosis exist in intra- and inter-specific hybrids and can it be predicted? (Chapter 5)

- Do hybrids exhibit complementary expression of parental traits? (Chapter 5)

- Evaluate hybrid performance to determine the environmental conditions under which it would be advantageous to plant such material. (Chapters 3 and 5)

c) Compare estimates of genetic parameters (including heritability, additive genetic correlation) and performance for various traits of progeny derived from OP and CP in *E. globulus* and *E. nitens*. (Chapter 3 and 4)

- Are genetic parameters derived from OP in *Eucalyptus* a reliable indicator of true additive genetic effects which are derived from CP? (Chapters 3 and 4)

- To what extent does inbreeding depression distort genetic parameter estimation using OP progeny, particularly from native stands? (Chapter 4)

d) Determine the proportion of additive and non-additive genetic effects on the performance of progeny in *E. globulus, E. nitens* and *E. nitens x globulus*. (Chapters 3, 4 and 5)

Wright (1964) recognized four stages in the process of getting successful F_1 hybrids into use -

1. Determine crossability (examined with in this Chapter);
2. Produce and test each combination (Chapters 3, 4 and 5);
3. Develop methods for mass producing planting stock;
4. Determining which particular trees will make the best parents.

Development of methods for mass producing planting stock, such as tissue culture and mass vegetative propagation, are beyond the scope of this project but have been investigated by others (Hetherington and Orme 1990; Wilson 1993; Borralho and Wilson 1994; England and Borralho 1995; Wilson 1996; Cotterill and Brindbergs 1997; Tibbits *et al.* 1997b; Wilson 1998; Wilson 1999; McComb *et al.* 2000). While the better parents have not been specifically identified in this thesis, the analysis of genetic parameters and subsequent production of breeding values for parents and progeny could be utilised for such identification.

Mating Design

In 1986 a survey of potential parents for the control-crossing program was carried out among forest owners in Tasmania to determine if the above objectives could be met. The survey revealed the following:

- About 40 unrelated seedlots of Toorongo provenance *E. nitens* had been used in plantations or trials prior to 1982. Trees less than five years old generally had little or no flower buds.

- There were only two sexually mature families of Northern NSW *E. nitens* and these trees were generally in poor health, thus it was not feasible to make inter-provenance crosses in this species.

- Suitable parent trees of *E. nitens* were scarce, as flower numbers per tree were generally low and trees were widespread. This led to a reduction in the size of the anticipated crossing program.

The selection of parent trees and mating design described below was used to take account of the available material and to meet the objectives of the project.

Eucalyptus globulus

Eight unrelated trees were chosen in the Woolnorth, Tasmania seedling seed orchard which was established in 1980 (Volker *et al.* 1990). The provenances of these trees were King Island 39°55″S 144°00″E (K with three trees - Ka, Kb, Kc), South Flinders Island 40°13″S 148°02″E (F with one tree - Fd) and Taranna 43°02″S 147°53″E (T with four trees - Te, Tf, Tg and Th) (Table 2.1). Locations of these provenances are detailed in Volker and Orme (1988). Open-pollinated seed was collected from each tree (collectively referred to as GSOP; Table 2.1).

Pollen was collected from widely separated trees in natural stands on King Island (10 trees) and Taranna (16 trees). Open pollinated seed was also collected from each tree (collectively referred to as GOP; Table 2.1).

Pollen from the natural stand trees was crossed with each of the eight female trees to undertake the 8 female X 26 male factorial mating design comprising intra- and inter-provenance hybrids (collectively referred to as GCP; Table 2.1). Cross types produced as intra-provenance were TT, KK and inter-provenance (or intra-specific) F_1 hybrids TK and KT (which were treated as one cross type in analyses).

Eucalyptus nitens

Eleven unrelated trees of Toorongo provenance (Pederick 1979) were chosen from two seedling seed orchards (SSO) and plantations. Three trees were located in Hampshire SSO (TO-k, TO-m, TO-n), four were in Huntsman SSO (TO-i, TO-l, TO-p, TO-s) and four were in plantations at Woolnorth (TO-r) and Highclere (TO-j, TO-o, TO-q).

The parents were mated in an incomplete half-diallel design (Namkoong 1979) where each parent is crossed with every other parent once and reciprocal crosses are not made (collectively referred to as NCP or NN; Table 2.1). Selfs were also attempted on each parent and open-pollinated seed (NOP) was collected from each tree.

Eucalyptus nitens x globulus

Eight of the above *E. nitens* (TO-i, TO-k, TO-l, TO-m, TO-n, TO-o, TO-p and TO-s) were used as female parents to produce inter-specific F_1 *E. nitens x globulus* hybrid families. At first an attempt was made to use all 26 *E. globulus* pollens on each female however, due to time constraints and low survival of capsules, effort was concentrated with a subset of ten pollens (T4, T6, T10, T11, T12, T15 from Taranna; K20, K22, K25, K26 from King Island; Table 2.1). The cross types of inter-specific F_1 hybrids was therefore *E. nitens* x Taranna *E. globulus* (NT) and *E. nitens x* King Island *E. globulus* (NK).

An initial study of pollination technique examined the possibility of using *E. globulus* as a female in hybrid crosses with *E. nitens*. We found that this cross was not possible due to unilateral incompatibility (Gore *et al.* 1990).

Table 2.1 Factorial mating design for crossing within *E. globulus* and between *E. nitens x E. globulus* with half-diallel mating design for crossing within *E. nitens*, indicating which families were used in field trials (*crosses attempted but not used in field trials are shown in italics*). The cross type of each family is indicated as intra-provenance crosses within *E. globulus* (TT and KK), inter-provenance crosses within *E. globulus* (TK, KT, FT, FK), inter-species F_1 hybrids (NT, NK) and crosses within *E. nitens* (NN) where the provenances of the parents and progeny are indicated by Taranna (T), King Island (K), Flinders Island (F) *E. globulus* and Toorongo (TO) *E. nitens*. GOP is native stand OP seed collected from the *E. globulus* trees which were used as male parents. GSOP is seed orchard OP seed collected from female *E. globulus* parents. NOP is OP seed collected from the *E. nitens* parent trees.

		E. globulus Males																									
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	K17	K18	K19	K20	K21	K22	K23	K24	K25	K26	OP
E. globulus Females	K-a	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KK	KK	KK	KK	KK	KK	KK	KK	KK	KK	GSOP
	K-b	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KK	KK	KK	KK	KK	KK	KK	KK	KK	KK	GSOP
	K-c	KT	KT	*KT*	*KT*	*KT*	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KT	KK	KK	KK	KK		KK	KK	KK	KK	KK	GSOP
	F-d	FT	FT	*FT*	FT	FT	FT	FT	FT	FT	FT	FT	FT	FT	FT	FT	FT	FK	FK	FK	FK	FK	FK	FK	FK	FK	FK	
	T-e	TT	TT	TT		*TT*	TT	TT	TT	TT	*TT*	TT	TT	*TT*	TT	TT	TT		TK		TK	*TK*	*TK*		*TK*	*TK*	*TK*	GSOP
	T-f	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TK	*TK*		*TK*	*TK*	*TK*	*TK*	TK	*TK*	TK	GSOP
	T-g	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TT	TK	TK	TK	TK	TK	*TK*	TK	TK	TK	*TK*	GSOP
	T-h	TT	TT	TT	TT	*TT*	TT	TT	TT	*TT*	TT	TT	TT	TT	TT	*TT*	TT		TK	TK	TK	TK	*TK*	*TK*	*TK*	TK	*TK*	GSOP

																														E. nitens Males							
OP		GOP	GOP		GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP		TO-k	TO-l	TO-m	TO-n	TO-o	TO-p	TO-q	TO-r
E. nitens Females	TO-i	*NT*	*NT*		*NT*	*NT*	*NT*	*NT*			*NT*	*NT*	*NT*			*NT*	*NT*	*NK*	*NK*	*NK*	*NK*	*NK*	*NK*	*NK*	*NK*	*NK*	*NK*	NOP	NN	*NN*	NN	NN	*NN*	NN	*NN*	NN
	TO-j																											NOP	*NN*	NN	NN	NN	*NN*	NN	NN	NN
	TO-k	*NT*	*NT*	*NT*	*NT*	*NT*	NT	*NT*	*NT*	*NT*	NT	NT	NT	*NT*	*NT*	*NT*	NT			*NK*	NK	*NK*	NK		*NK*	*NK*	NK	NOP		NN	NN		NN	*NN*	NN	NN
	TO-l				NT		NT					*NT*							*NK*	*NK*	NK	*NK*	NK	*NK*	*NK*	*NK*	NK	NOP			NN	NN	NN	NN	NN	NN
	TO-m	NT	*NT*	*NT*	NT	*NT*	NT	*NT*	*NT*	*NT*	NT	NT	*NT*			NT		*NK*	*NK*	*NK*	NK	*NK*	NK	*NK*	*NK*	NK	NK	NOP				NN	*NN*	NN	NN	NN
	TO-n	*NT*	*NT*	*NT*	NT	*NT*	NT	*NT*	*NT*	*NT*	*NT*	NT	NT	*NT*	*NT*	*NT*	*NT*	*NK*	*NK*	*NK*	NK	*NK*	NK	*NK*	*NK*	*NK*	NK	NOP					NN	*NN*	NN	NN
	TO-o			*NT*	NT	*NT*	NT				NT	NT	NT	NT	*NT*	NT					NK		*NK*				NK	NOP			NN			NN	NN	NN
	TO-p	NT				NT																						NOP							NN	NN
	TO-q																											NOP								*NN*
	TO-r																											NOP								
	TO-s							NT																												

Control Pollination Techniques

Pollen extraction and handling

Potential parent trees were monitored throughout the flowering season. Tree branches carrying flowers were collected a few days prior to anthesis. Open flowers and all leaves were removed from the branch immediately. The branches were placed in water and covered to prevent contamination. In the laboratory, branches were recut under water to improve longevity. Flowers were removed for pollen extraction each day as anthesis occurred.

The "wet" pollen extraction method (Griffin *et al.* 1982a) proved to be unsatisfactory for these species. Pollen was extracted from *E. nitens* and *E. globulus* by a "dry" method (Potts and Marsden-Smedley 1989). In this method branches with enclosed flowers, *i.e.* still covered with an operculum, were collected immediately prior to anthesis. Open flowers were removed from the branch at this time. The branches were placed in water and isolated from other material in the laboratory. About 4 hours after anthesis the flower was removed and placed in a desiccator for 12 to 24 hours. The flowers were then shaken and brushed to remove the pollen onto a smooth surface.

Pollen was stored in size "OO" gelatin capsules in 50ml airtight vials filled with silica gel. The vials were stored in a commercial freezer at -18°C. A single gelatin capsule was removed from the freezer when required and transferred to a 5ml vial for transport to the field or temporary storage in a refrigerator at 4°C. Vials were transported to the field in a small ice box at ambient temperature. Ice was added to the box to act as a coolant when day-time temperatures were expected to exceed 25°C as the gelatin capsules tend to melt in warmer weather.

Frozen pollen (-18°C) can be stored for at least 12 months with little loss of viability, although it proved difficult to test *in vitro* (see below). It was possible to store fresh pollen at room temperature or a commercial refrigerator at about 4°C for at least eight weeks with little or no loss in viability provided the pollen is in a moist free environment such as a desiccator.

Pollen germination testing

Pollen was tested throughout the pollination season by *in vitro* and *in vivo* methods to determine its viability. Test methods are described in Griffin *et al.* (1982a).

In vitro pollen germination tests in *Eucalyptus* species were examined in detail by Potts and Marsden-Smedley (1989). Germination percentages above 50% were rare and in most cases germination rates of about 20 to 30% were found with fresh pollen. In the present program pollen was discarded when *in vitro* tests revealed a germination rate of less than 5%.

The *in vivo* test is a reliable but destructive means of examining pollen germination on the stigma and pollen tube growth in the style (Sedgley *et al.* 1989; Sedgley and Smith 1989; Gore *et al.* 1990; Egerton-Warburton *et al.* 1993). Using this method we were able to confirm *in vitro* tests and determine reasons for failure of crosses. The unilateral incompatibility of *E. globulus x nitens* was examined using this method (Gore *et al.* 1990).

Self-pollination

Self pollination was attempted using two methods. In 1987/88 un-emasculated flowers were bagged prior to anthesis and blow-fly pupae, produced by CSIRO Division of Entomology, were placed in the bag. This method has been used successfully in the past (Pryor and Boden 1962). In 1988/89 selfed seed was produced by isolating unopened flowers as described above. After most

flowers in the bag had opened, anthers from other isolated flowers were used to brush pollen onto the stigmata. In addition, some flowers were allowed to develop without flies or manipulated pollination. Seed was produced by all three techniques. However, there were insufficient quantities of seed or later, seedlings to establish in the field trials in most cases. The effects of selfing and varying levels of inbreeding on performance and genetic evaluation of eucalypts have been examined in detail elsewhere (Pryor 1978; Potts *et al.* 1987; Griffin 1990; Hardner and Potts 1995a; Hardner *et al.* 1995; Hardner 1996).

Manipulated cross-pollination

A three-visit pollination technique was used for cross pollination of *E. globulus* and *E. nitens* as follows:

1. Anthesis (day 1) was defined as the time when the operculum was beginning to lift. Flowers were emasculated at anthesis by use of a scalpel (*E. globulus* and *E. nitens*) or modified electrician's wire strippers (*E. nitens*). All anthers were removed. In *E. nitens* flowers which were not ready for emasculation were removed from the umbel. Excess leaves were removed from the branch so that the bag would not be crowded with leaves, leading to increased humidity in the bag.

2. Bags or sleeves made from unwoven Terylene were fixed over the branch by use of electrical wire ties.

3. At day 7, for both *E. globulus* and *E. nitens*, bags were removed temporarily for pollination. Pollen was applied to the stigma by use of a matchstick. Unlike *E. regnans* (see Griffin *et al.* 1982a) no observable swelling of the style occurred, although stigmatic

exudate was encountered with *E. globulus* at the time of peak receptivity. Nectar flow was observed at this time. A discussion on the effect of pollination date on seed yields in *E. nitens* is given in Tibbits (1989). Bags were replaced after pollination.

4. Bags were removed permanently approximately three to four weeks after pollination. At this stage styles had abscised from flowers.

It has now been confirmed that a cut style technique for single visit pollination in *E. globulus* is feasible due to the robustness of the flower to withstand damage and adequate pollen germination in the absence of exudates normally found on the stigma (Harbard *et al.* 1999; Williams *et al.* 1999). It is apparent, from the work completed in this study, that *E. nitens* flowers are very sensitive to damage to the style prior to fertilisation and such a technique may not be possible with this species.

Capsule and Seed Yields

Eucalyptus globulus

In 1987/88 the average number of intact seeds per capsule ranged from 22 to 81 for the eight female parents (Table 2.2). In 1988/89 severe predation by seed-eating larvae of *Megastigmus* spp. wasps severely reduced the average seed yield in six of the eight trees to an overall range of 6 to 52 (Table 2.2). Over the two years, there was an average of 38 seeds per capsule (Table 2.3) for control-pollination, which compares with an average yield of about 20 seeds per capsule for open-pollination (Tibbits 1991; Griffin *et al.* 1993).

Table 2.2 Capsule survival (pollination to harvest) and intact seed yield per capsule for inter- and intra-provenance hybrids of *Eucalyptus globulus* completed over two pollination seasons (1987/88 and 1988/89).

Female	Taranna pollen				King Island pollen			
	Capsule survival (%)		Seed per capsule (number)		Capsule survival (%)		Seed per capsule (number)	
	87/88	88/89	87/88	88/89	87/88	88/89	87/88	88/89
K-a	80.8	92.5	48.1	47.3	70.5	100.0	62.8	51.6
K-b	61.4	91.5	33.3	34.3	66.2	85.7	44.3	24.1
K-c	31.4	81.5	52.3	5.6	61.0	75.0	45.1	10.1
K average	43.7	66.6	33.7	22.1	49.7	65.4	38.3	21.7
F-d	100.0	79.2	47.0	14.6	62.2	50.0	58.0	22.5
T-e	56.8	25.0	22.1	13.5	39.5	27.8	21.5	6.4
T-f	69.0	46.2	58.1	12.2	-	38.5	-	14.8
T-g	70.2	55.6	80.6	34.6	-	31.9	-	29.4
T-h	55.7	24.0	43.2	12.0	85.7	6.5	45.0	8.5
T average	62.9	37.7	51.0	18.1	62.6	26.2	33.3	14.8

Capsule survival at harvest averaged 61% of flowers pollinated, over the two seasons (Table 2.2). There was no relationship between capsule survival and seed yield per capsule. The *Megastigmus* spp. attack in 1988/89 markedly reduced seed yield per capsule (Table 2.2).

Twelve of 209 crosses attempted, failed to produce seed (Table 2.4). The majority of these failed crosses occurred with King Island pollen mated with Taranna females (TK), resulting in average of 11.4 seeds harvested per flower pollinated compared with an average of 25.4 for the reciprocal inter-provenance cross (KT; Table 2.4). This may be confounded with the seed predation as most K pollens were applied to T females only in 1988/89. Inter-provenance crosses involving the single Flinders Island female produced an

average of 14 and 38 seeds per flower pollinated for FT and FK respectively (Table 2.4).

Selfing reduced capsule survival to 18% and yielded an average of only seven seeds per capsule (Table 2.3). Only three out of eight selfs produced seed (Tables 2.1 & 2.4).

Table 2.3 Pollination success and seed production for a range of control pollinated and self cross types in *E. globulus, E. nitens x globulus* and *E. nitens* across both seasons of pollination.

Cross type	Number of flowers pollinated	Number of capsules harvested	Capsules harvested per flower pollinated (%)	(Range)	Number of seeds produced	Seeds per capsule harvested	(Range)	Seeds per flower pollinated
E. globulus	1,222	749	61	(26-79)	28,798	38.4	(27-78)	23.6
E. globulus self	120	22	18	-	155	7	-	1.3
E. nitens x globulus	3,579	838	23	(5-51)	3,084	3.7	(2.1-5.8)	0.9
E. nitens	3,571	1,052	29	(14-49)	6,772	6.4	(3.3-7.7)	1.9
E. nitens self	848	84	10	-	103	1.2	-	0.1

The confounding effects of season, different operators, and insect predation to name a few, make it difficult to interpret pollination success and seed yield data any further in relation to fecundity of male or female parents used in this program.

Eucalyptus nitens x globulus

The hybrids produced an average of 3.7 seeds per capsule (range 2.1 to 5.8) with an average capsule survival of 23% (Table 2.3). 99 of 122 crosses produced seed (Table 2.4), although some of these crosses produced very small quantities, which were not used in the subsequent field trials, leaving 35

families for field trial evaluation (Table 2.1). 25 of 73 (34%) of *E. nitens x globulus* (NT) produced sufficient material for field trials compared with 10 of 49 (20%) in NK (Table 2.1).

Eucalyptus nitens

The *E. nitens* outcrosses produced an average of 6.4 seeds per capsule harvested (range 3.3 to 7.7) (Table 2.3) with 2.1 seeds per flower pollinated (Table 2.4), reflecting low flower survival to capsules of 29%, with a range 14 to 49% (Table 2.3). All of the 44 crosses attempted produced seed (Table 2.4), however nine crosses were excluded from field trials (Table 2.1) due to low seedling numbers.

Selfs succeeded in producing seed in six out of ten attempted. The average of 1.2 seeds per capsule and 9.9% capsule survival (Table 2.3) was considerably lower than either hybrid or outcross yields.

Table 2.4 Average number of seed harvested per flower pollinated for *E. globulus* Taranna pollen crossed with *E. globulus* King Island (K), Flinders Island (F), Taranna (T) and *E. nitens* (TO) females, including selfs. Overall averages (Ave.) are given for provenance effects on each parent.

		E. globulus male																											*E. nitens* male									
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	Ave. T	K17	K18	K19	K20	K21	K22	K23	K24	K25	K26	Ave. K	SELF									Ave. N
	K-a	42.0	25.0	49.4	56.8	30.2	44.6	47.2	48.3	40.8	40.3	45.1	28.9	36.6		43.6	52.5	42.1	28.8	25.0	0.0	40.0	66.3	58.8	87.8	40.0	40.8	57.5	44.5	1.0									
	K-b	44.5	5.7	35.8	28.6	18.4	14.0	12.5	40.0	39.1	42.2	12.0	17.3	26.7	17.1	27.8	23.2	25.3	33.1	40.3	21.7	25.8	3.4	15.3	45.0	47.1	61.2	61.0	35.4	9.0									
	K-c	5.0	1.7	2.7	1.1	3.0	53.8	62.0	10.4	3.2	1.7	5.5	2.5	4.3	5.1	4.6	7.2	10.9	2.5	46.0	28.7	12.5		50.0	39.5	26.6	15.0	50.0	30.1	0.0									
	Ave. K	30.5	10.8	29.3	28.8	17.2	37.5	40.5	32.9	27.7	28.0	20.9	16.2	22.5	11.1	25.3	27.6	25.4	21.5	37.1	16.8	26.1	34.9	41.4	57.4	37.9	39.0	56.2	36.8	3.3									
E. globulus female	F-d	7.5	5.0	3.5	7.3	6.0	33.5	10.0	6.8	11.0	16.7	34.0	5.3	47.0	10.0	2.7	11.5	13.6	5.6	36.5	63.0	26.7	13.8	48.7	44.0	77.3	29.5	34.8	38.0	0.0									
	T-e	23.0	18.1	25.2	1.4	6.0	11.4	19.4	5.0	16.2	3.5	14.7	12.9	1.1	3.9	14.8	15.3	12.0		3.7		7.5	0.5	2.0		1.5	6.0	2.0	3.3	0.0									
	T-f	26.4	14.5	70.0	33.3	35.8	46.5	46.9	11.3	45.7	25.8	59.8	41.4	17.7	40.7	6.7	50.0	35.8	16.7	3.0		0.0	0.0	3.3	0.0	17.3	0.0	6.7	5.2	0.0									
	T-g	100.0	38.8	45.0	68.7	35.8	52.0	68.0	73.0	66.7	60.7	71.3	100.0	45.5	16.4	51.6	17.0	56.9	33.8	12.3	7.6	17.6	7.5	3.8	11.0	5.0	4.0	0.0	10.3	0.0									
	T-h	45.5	4.7	46.7	29.5	0.0	42.9	24.5	5.4	0.0	8.3	41.3	5.7	25.3	22.4	0.0	29.8	20.8	0.0	56.0	50.0	33.3	55.5	0.0	0.0	1.8	8.6	0.0	20.5	4.0									
	Ave. T	48.8	19.0	46.7	43.8	19.4	38.2	39.7	23.7	32.1	24.6	46.8	40.0	22.4	20.8	18.3	28.0	32.0	16.8	18.7	28.8	14.6	15.9	2.3	3.7	6.4	4.7	2.2	11.4	1.0	TO-j	TO-k	TO-l	TO-m	TO-n	TO-o	TO-p	TO-q	TO-r	
	TO-i	0.0	0.0		0.2	0.8	0.3	0.4			0.1	0.2	0.0			0.3	0.4	0.2	2.2	1.2	0.2	0.4	0.1	0.0	0.0	0.0	0.2	0.1	0.4	0.4	1.8	0.6	1.1	0.2	0.6	0.5	1.2	0.7	3.2	1.1
	TO-j																		0.0										0.0	0.0		0.3	2.0	1.8	1.6	1.4	0.8	1.9	0.6	1.3
	TO-k	0.1	0.4	0.0	0.5	0.4	1.5	1.3	2.3	0.6	2.3	2.1	1.2	0.2	2.1	1.5	2.7	1.2			2.3	1.6	0.9	5.0		0.0	0.1	3.1	1.9	0.2			4.0	5.5		6.3	4.2	3.3	3.2	4.4
	TO-l				0.7		1.7					2.1						1.5		0.7	0.0	4.6	0.5	3.1	1.5	1.5	1.1	1.2	1.6	0.0				3.0	1.8	1.8	3.0	3.7	2.9	2.7
	TO-m	3.1	2.0	0.0	2.5	0.2	1.1	0.3	0.0	0.0	0.8	1.2	1.0			1.8		1.1	0.0	0.0	0.0	3.5	0.0	0.5	0.2	0.2	1.4	6.0	1.2					2.5	1.7	1.8	2.3	1.7	2
E. nitens female	TO-n	0.1	0.3	0.0	0.7	1.1	3.0	3.0	0.0	0.6	0.3	2.4	2.6	2.6	0.4	1.7	1.7	1.3	0.1	0.0	1.4	0.4	0.7	0.0	0.3	0.2	0.4	0.2	0.4					0.1	2.6	1.0	1.2	1.0	1.2
	TO-o			0.5	2.1	0.0	0.3				0.9	2.5	1.5	0.0	0.9	0.9		1.0				1.6		0.0				2.8	1.5	0.2				9.2			2.7	1.2	2.4	3.9
	TO-p	2.9				2.0																								0.1							1.6	4.3	2.9
	TO-q																													0.0								0.6	0.6
	TO-r																													0.0									
	TO-s							5.6										5.6																					
	Ave.	1.2	0.7	0.1	1.1	0.7	1.3	2.1	0.8	0.4	0.9	1.8	1.3	0.9	1.1	1.2	1.6	1.1	0.6	0.5	0.8	2.0	0.4	1.4	0.5	0.4	0.6	2.2	1.0	0.1	1.8	0.5	2.4	3.9	1.3	2.4	2.1	2	2.2	2.1

Factors influencing seed yield and capsule survival

Flower survival in *E. nitens* appears to be affected by a number of non-genetic factors including stage of development at time of emasculation, physical damage to style or hypanthium during pollination operations, weather conditions at time of pollination, health of branch carrying flowers, development of embryos.

We found that developing *E. nitens* capsules will abort within four weeks of pollination if physical damage occurs or fertilization is inhibited. Generally all capsules pollinated in mid-summer, which were retained by late autumn, would survive to harvest in the following summer.

E. globulus capsules appear to be more robust and less sensitive to damage during pollination. Abortions generally occurred by late autumn. However insect predation by *Megastigmus* spp. did not produce consistent abortion of flowers or capsules but did have a significant impact on the proportion of capsules harvested and intact seed yield per capsule.

In both species seedless capsules were never harvested, although capsules with empty seed (lack of embryo) had developed normally.

Establishment of Field Trials

Trial sites

Field tests were planted over a range of environments throughout southern Australia (Table 2.5). In Tasmania, sites were chosen in the commercial planting zones for *E. globulus* or *E. nitens*. A "rule of thumb" for commercial planting of *E. globulus* in Tasmania is that plantations are not made above 300m, where *E. nitens* is preferred, however this may vary with site

characteristics such as soil type, frequency and severity of winter frosts. In the case of West Ridgley (WR), the site was seen to be at the limit of the range where *E. globulus* could be planted successfully in particular due to experience with winter frosts in the area. The expense of producing a small amount of hybrid material limited our ability to test performance in a more severe environment as severe mortality in the first years after establishment would have limited the potential evaluation of growth and other later-age traits. The area is within a well-established zone of *E. nitens* plantation. This type of site is likely to be most favoured for the use of hybrids in future. At Hampshire (HA), a small trial was established to test the performance of a small number of hybrids at high altitude where *E. nitens* would be the only species planted and *E. globulus* would be unlikely to survive. All other Tasmanian sites fall within areas where *E. globulus* has been planted successfully or grows naturally. Some sites in Victoria and Western Australia fall within current (FL and WA) *E. globulus* plantation sites. MA and BE sites are well outside the accepted planting range for *E. globulus* ssp. *globulus*, although they are close to the natural range of *E. globulus* ssp. *bicostata* and *E. globulus* ssp. *maidenii* respectively. Due to limitations in seed numbers, each trial varies in size and number of seedlots. *E. nitens x globulus* hybrids were concentrated at WR with a small number of families at HA and FL.

Table 2.5 Location of field trials.

Site, State	Site Code	Latitude	Longitude	Altitude (m)
West Ridgley, Tasmania	WR	41°09'S	145°46'E	160
Boyer, Tasmania	BO	42°47'S	141°06'E	15
Hampshire, Tasmania	HA	41°15'S	145°45'E	470
Franklin, Tasmania	FR	43°04'S	146°53'E	370
Parkham, Tasmania	PA	41°26'S	146°37'E	200
Flynn, Victoria	FL	38°18'S	146°40'E	170
Mansfield, Victoria	MA	36°55'S	146°14'E	950
Bega, New South Wales	BE	36°36'S	149°46'E	120
Manjimup, Western Australia	WA	34°12'S	116°01'E	240

Seedlings were grown at three nurseries - Ridgley, Tasmanian for five field trials (WR, BO, FR, HA, PA), Traralgon, Victoria for three field trials (FL, MA, BE) and Manjimup, Western Australia for one field trial (WA).

Seedlots were allocated to each site according to availability during nursery production (Table 2.6). Some families produced in the crossing program, particularly selfs in both species and some hybrid families did not produce sufficient numbers of seedlings for field establishment.

Table 2.6 The number of families within each cross type planted in each field trial.

Cross Type		Trial sites									Total number of families
		WR	BO	HA	PA	FR	FL	MA	BE	WA	
GCP	TT	55	8		50	32	38	22	40	35	57
	KK	28	4		27	18	24	13	19	21	28
	TK	17			9	4	6	2	4	5	18
	KT	43	8		33	17	32	15	25	27	44
	FT	15			6		3	1	1	3	15
	FK	10			10	2	8	2	3	5	10
	Total	**168**	**20**		**135**	**73**	**111**	**55**	**92**	**96**	**172**
GOP	T	14	3	14	14	12	13	7	11	13	15
	K	10	5	9	8	9	8	6	8	8	10
	Total	**24**	**8**	**23**	**22**	**21**	**21**	**13**	**19**	**21**	**25**
GSOP	T	4	1	3	4	2	3	3	3	3	4
	K	3		3	3	2	3	2	2	3	3
	F	1	1	1	1	1	1	1	1	1	1
	Total	**8**	**2**	**7**	**8**	**5**	**7**	**6**	**6**	**7**	**8**
HYB	NT	24		2			5				25
	NK	10		2			1				10
	Total	**34**		**4**			**6**				**35**
NCP	NN	**35**		**16**	**1**	**9**	**32**		**18**		**40**
NOP	NOP	**9**		**8**	**8**	**6**	**9**	**8**	**9**	**5**	**9**
Total number of families		**278**	**30**	**58**	**174**	**114**	**186**	**82**	**144**	**129**	**289**

Trial design

The trials were designed using the ALPHAGEN program (SASS 1987). ALPHAGEN generates incomplete block designs of the alpha-lattice type

(Paterson and Williams 1976). The design parameters are shown in Table 2.7. The aim was to use 4 replicates per site and to keep the incomplete block sizes as small as possible to reduce environmental variability within blocks. The availability of material precluded larger plot sizes, so five tree line plots were utilised on most sites.

Table 2.7 Experimental design at each trial site.

Experimental design parameters	Trial Sites								
	WR	**BO**	**HA**	**PA**	**FR**	**FL**	**MA**	**BE**	**WA**
Replicates	4	4	4	4	4	4	4	4	7
Blocks per replicate	15	1	7	13	9	11	11	10	11
Plots per block	20	30	9	14	13	18	8	14	11
Trees per plot	5	10	5	5	5	5	5	5	3

A constraint was placed on the trial design in that breeds were to be kept together in the field to minimize effects of competition and difference in growth habit between different breeds (*i.e. E. globulus* vs. *E. nitens* vs. *E. nitens x globulus*). This constraint was not used in the HA trial. For example, at WR where there are 15 plots per incomplete block, each block consists of 9 adjoining plots of *E. globulus* and 3 plots each of *E. nitens x globulus* and *E. nitens*. Within each breed the cross types were allocated randomly.

All trials, except BO and WA, were arranged in five tree plots with four replicates per site. The BO plots were two rows of five tree plots and the WA site had three tree line plots. The number of plots per incomplete block varied according to numbers of seedlots per breed at each site.

Site preparation and planting

All sites except WA were prepared for planting in late winter 1990. WA was established in 1991.

Sites preparation consisted of pre-plant weed control followed by ripping and mound plough cultivation. Weed regrowth was monitored in the first year after planting and secondary weed control was carried out if necessary. Spacing at the sites was about 3 x 2.5 metres (1333 stems per hectare) for Tasmanian sites (WR, BO, HA, PA, FR) and 3 x 3 metres (1111 stems per hectare) at other sites.

Early survival

Survival at most sites was good. However, Mansfield site was severely affected by snow in the first year and Bega was almost completely defoliated by insects in the second year. These sites were not considered for analysis in this study.

Assessment Schedule

Seedling material was assessed in the Ridgley nursery for frequency of abnormalities, this data was reported in Potts *et al.* (1992).

Seedlings were sampled from the Ridgley nursery at about age 6 months for assessment of frost resistance by the electro conductivity of leachate method (reported in Volker *et al.* 1994 and Chapter 3).

Most sites were assessed for diameter at breast height over bark (dbhob) in Years 2, 3, 4 and 6 years from planting. The West Ridgley site was also assessed for dbhob at year 10. Year 2 diameter and height assessment was carried out at all sites. Some of this data was analysed and reported in Hodge

et al. (1996) and Chapter 4 (diameter only). A more detailed assessment schedule is presented in Chapter 4.

The West Ridgley trial was also assessed for damage by *Mycosphaerella* spp. leaf spot fungi at age 3 years (Dungey *et al.* 1997), time of vegetative phase change and first flowering (Jordan *et al.* 1999; Jordan *et al.* 2000) which were not part of the study reported in this thesis.

Chapter 3: Genetic variances and covariances for frost tolerance in *Eucalyptus globulus*, *E. nitens* and their F_1 hybrid

Introduction

Temperate eucalypts are widely used as a source of pulp and paper manufacture in the cool-temperate regions of the world but further extension of their planting is generally limited by seasonal low temperatures and occasional frosts. Temperate eucalypt species which have been shown to be relatively frost hardy such as *Eucalyptus nitens, E. gunnii, E. delegatensis, E. viminalis* and *E. dalrympleana* tolerate temperatures as low as -12°C provided acclimation has taken place before the onset of frost (Paton 1981; Davidson and Reid 1987; Tibbits and Reid 1987b). More sensitive temperate species include *E. globulus* and *E. regnans*, with critical temperatures around -7°C (Turnbull and Pryor 1984; Hallam and Reid 1989; Eldridge *et al.* 1993).

E. globulus and *E. nitens* are two of the most important temperate eucalypt species, due to their excellent productivity, fibre characteristics and pulp yield. They are currently being established in large areas in Australia, South America and south-western Europe. *E. nitens*, the more frost tolerant of the two, has become the preferred species for eucalypt plantation forestry in higher altitude areas, subject to frequent winter frosts, whereas *E. globulus* has been confined to milder sites.

Improvement in frost tolerance has the advantage of significantly increasing the area of land suitable for eucalypt plantations by reducing the risk of loss

due to frost. There have been numerous studies of frost tolerance with eucalypts but these have largely been confined to *in situ* observation or comparison of OP material in provenance or family trials in the field (Ashton 1958; Awe and Shepherd 1975; Harwood 1980; Rook *et al.* 1980; Wilcox *et al.* 1980; Griffin *et al.* 1982b; Wilcox 1982; Burgess 1983; Darrow 1983; Evans 1983; Higa 1986; Tibbits and Reid 1987a; Hallam and Reid 1989; Marcó *et al.* 1991; Raymond *et al.* 1992b). Evidence of provenance differences in frost tolerance have been reported for *E. nitens* (Tibbits and Reid 1987a; Hallam and Tibbits 1988; Raymond *et al.* 1992b; Tibbits and Hodge 2001) and for *E. globulus* (Almeida *et al.* 1995) but studies on the magnitude of additive genetic variation have been limited. Frost damage in leaves and branches of field grown trees of open-pollinated origin has been shown to be highly heritable in *E. nitens* (Tibbits and Reid 1987a) with narrow-sense heritability (h^2) above 0.6 but low to moderate in *E. grandis* (van Wyk 1976) and *E. gunnii* (Cauvin *et al.* 1993) with h^2 between 0.10 and 0.29. More recently, indirect methods for frost tolerance assessment, based on electric resistance (or conductivity) of leaf exudates have been developed (Raymond *et al.* 1986). They have the advantage of being non-destructive and provide a continuous variable, more suitable to statistical treatment and interpretation (reviewed by Raymond et al.1992a). The inheritance of electro conductivity measurements has been investigated in *E. nitens* (Tibbits and Reid 1987a; Raymond *et al.* 1992b; Tibbits and Hodge 2001), in a small number of inter-specific F_1 hybrids including *E. nitens x globulus* (Tibbits *et al.* 1991a), and *E. regnans* (Raymond *et al.* 1992a) and have shown a high heritability, with values usually above 0.6.

Although the economic importance of tolerance to frost damage is obvious, basic information on the genetic control of this trait in eucalypts, and in particular in *E. nitens* and *E. globulus* and their F_1 hybrid is required. This study reports genetic parameters of frost damage at three test temperatures,

based on electro conductivity measurements in both open-pollinated (OP) and control-pollinated (CP) material for these three populations.

Materials and methods

Mating designs in *Eucalyptus globulus*

The *E. globulus* plants tested for frost tolerance were derived from a subset of the incomplete factorial crossing described in Chapter 2 (Table 2.1) involving 19 unselected parents from three provenances (Table 3.1), namely Taranna, SE Tasmania (with 11 parents), King Island (7) and Flinders Island (1). In total, 580 seedlings from 58 full-sib families (10 per family) from the incomplete 8 x 11 factorial were assessed. Open-pollinated progeny (GOP) came from the same 11 parents used as males in the factorial mating, plus three extra Taranna parents used in inter-specific hybrids described below (Table 3.1). Open-pollinated progeny from the eight female parents (GSOP) were derived from seed collected from those trees, which were located in a multi-provenance seedling seed orchard (Table 3.1). See Chapter 2 for a more complete description of the OP material.

Mating design in *Eucalyptus nitens*

The control-pollinated *E. nitens* plants (NCP) were derived from an almost complete half diallel mating (see Table 3.1) of 10 first generation parents (see Chapter 2 for more detail). In total, 340 seedlings from 34 full-sib families (10 seedlings per family) from an almost complete 10 x 10 half-diallel were assessed. Open-pollinated progeny was also obtained from 9 parents used in the half diallel.

Mating design in *Eucalyptus nitens x globulus*

The control-pollinated *E. nitens x globulus* plants were derived from a factorial mating design involving 14 of the male *E. globulus* parents described above and 6 of the *E. nitens* from above which were used as females (Table 3.1). In total 400 seedlings from 40 full-sib families were assessed. 28 families had Taranna (NT) and 14 families had King Island (NK) *E. globulus* male parents.

Plant material

The seedlings of both *E. nitens, E. globulus* and *E. nitens x globulus* were raised in North Forest Products' East Ridgley nursery in northern Tasmania. In January 1990 seed was germinated in flat trays filled with alluvial soil under controlled light and temperature conditions. Approximately three weeks after germination when seedlings were still in the cotyledonary stage they were pricked out into paper pots of 4cm diameter and 10cm depth. The seedlings were then moved outdoors, under shade for a further ten weeks. In April the seedlings were transplanted into a field nursery with formed beds. Families were planted as lines with four families across each bed. Spacing within the beds was approximately 5cm within lines and 10cm between lines. Families were randomly allocated in the nursery as unreplicated line plots. A control seedlot of *E. globulus* was placed in 10 locations throughout the nursery to test for environmental variation.

Sampling strategy and frosting techniques

Measurements of frost damage were taken at three test temperatures: -5.5, -7.0 and -8.5^{o}C (referred to hereafter as traits RC5, RC7 and RC8 respectively). Four leaf discs per test temperature were sampled from each of 10 seedlings per family following guidelines set by Raymond *et al.* (1992a). Families were planted in the nursery in population groups, *i.e. E. nitens* CP and OP, *E.*

globulus CP and OP, *E. nitens x globulus*. Within each group there was a random allocation of families regardless of cross type. The ten seedlings within each family were chosen at random. The families chosen for testing were pre-determined and sampling was carried out such that a number of families from each group were tested at the same time. Two leaves were collected from each plant, one from the youngest fully expanded leaf pair and the other from the leaf pair directly below following the method of Raymond (1986). Two discs from each of the two leaves per seedling were frosted at each temperature. The testing was carried out over a two-week period in July 1990. The control seedlot was sampled every four days throughout the testing period and no changes in relative conductivity were detected.

The screening for frost tolerance was based on electrical conductivity methods. The equipment consisted of plexiglass baths containing aqueous ethylene glycol solution in which racks of test tubes were suspended (Raymond *et al.* 1986). Cooling to -5.5, -7.0 and -8.5°C was achieved by pumping liquid at -30°C from a refrigerated bath through a pressure regulated copper coil in the test baths with temperature regulated by a thermomix.

Table 3.1 Open-pollinated (OP) and control-pollinated (CP) families tested for frost damage from a factorial mating in *E. globulus* and *E. nitens x globulus* and partial diallel in *E. nitens*. Provenances are Taranna (T), King Island (K), and Flinders Islands (F) in *E. globulus* and Toorongo (TO) in *E. nitens*. Cross types for each family included are indicated by annotation (KT, KK, FT etc.). GSOP is from OP seed collected from trees in a seedling seed orchard, which were used as females, GOP is from OP seed collected from trees in natural stands, which were used as males, and NOP is OP seed collected from *E. nitens* parents. NT and NK are the F1 hybrid families of *E. nitens x globulus* with Taranna and King Island male parents respectively.

		E. globulus males														OP
		T1	T4	T5	T6	T7	T10	T11	T12	T13	T15	K20	K22	K25	K26	
E. globulus females	Ka		KT	KT	KT		KT	KT	KT		KT	KK	KK	KK	KK	GSOP
	Kb		KT		KT		KT	KT	KT		KT	KK		KK	KK	GSOP
	Kc				KT			KT			KT	KK	KK	KK	KK	GSOP
	Fd		FT		FT		FT	FT				FK	FK	FK	FK	GSOP
	Te				TT			TT	TT		TT	TK				GSOP
	Tf		TT		TT		TT	TT	TT							GSOP
	Tg		TT		TT		TT	TT	TT		TT	TK		TK		GSOP
	Th				TT		TT	TT	TT					TK		GSOP

OP		GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP	GOP		*E. nitens* males							
																	TO-k	TO-l	TO-m	TO-n	TO-o	TO-p	TO-q	TO-r
E. nitens females	TO-i															NOP	NN		NN	NN		NN		NN
	TO-j															NOP		NN	NN	NN		NN	NN	NN
	TO-k				NT		NT	NT	NT			NK			NK	NOP		NN	NN		NN		NN	NN
	TO-l		NT		NT			NT				NK	NK		NK	NOP			NN	NN	NN	NN	NN	NN
	TO-m	NT	NT		NT		NT	NT			NT	NK		NK	NK	NOP				NN		NN	NN	NN
	TO-n		NT		NT			NT	NT	NT	NT	NK			NK						NN		NN	NN
	TO-o		NT		NT		NT	NT	NT		NT	NK			NK	NOP			NN				NN	NN
	TO-p	NT		NT												NOP							NN	NN
	TO-q															NOP								
	TO-r															NOP								
	TO-s					NT																		

Single 8mm diameter leaf discs were placed in separate test tubes and racks of tubes were placed in test baths at 2.0°C. Bath temperature was lowered at 4°C per hour to -2.0°C when 0.1g of finely crushed ice was added such that it was in contact with the leaf disc to prevent super cooling. The bath temperature was then lowered to the required minimum (-5.5, -7.0 or -8.5°C) and held at this temperature for one hour. The racks of tubes were then removed and placed in a refrigerator at 3°C for a 24-hour post-frost recovery period. Two millilitres of deionised distilled water were then added to each test tube and the racks were allowed to stand for a further 24 hours at room temperature. Electrical conductivity of leachate from each sample (denoted **ct**) was then measured. Racks of tubes were then immersed in a hot water bath at 80°C for 10 minutes. After standing for a further 24 hours at room temperature, conductivity of leachate (denoted **ck**) was remeasured and this was assumed to be the absolute maximum conductivity. The three test temperatures were conducted simultaneously as there were three baths available. The degree of damage sustained by the leaf tissue was assessed using a relative conductivity value (RC) calculated as (Raymond *et al.* 1986):

$$RC = \sqrt{(ck - ct)/ck} \qquad \text{(Equation 3.1)}$$

Statistical analyses

An initial analysis of residuals (not presented) based on individual disc RC measurements was carried out to locate and remove outlier observations after checking each test temperature. These outliers were generally where the **ct** value exceeded the **ck** value or they were so close together as to indicate that the freezing methodology was not effective. In the most extreme case 4% of observations were removed. Once these outliers were removed, a mean value

for each seedling was calculated and used in the genetic analysis. Overall means and standard errors were calculated for each cross type.

Mid-parent heterosis ($H_{1,2}$) was estimated as the deviation of the hybrid from the mid-parent value (%) derived from the cross type means as:

$$H_{1,2} = \left[\left(F_1 - \left((P_1 + P_2)/2\right)\right) \div \left((P_1 + P_2)/2\right)\right] \times 100 \quad \text{(Equation 3.2)}$$

where F_1 is the mean RC value for the hybrid (intra- or inter-specific), P_1 and P_2 are the means for parent 1 and 2 cross types respectively.

Inbreeding depression (ID) was estimated as the deviation of the open-pollinated cross type mean from the control-pollinated cross type mean as:

$$ID(\%) = \left[\frac{CP - OP}{CP}\right] \times 100 \quad \text{(Equation 3.3)}$$

The tests of significance of the deviation of the F_1 from the mid-parent value and of differences between fixed cross types were undertaken using a mixed model where family within cross type was the error term. These tests were undertaken with the contrast option in PROC MIXED of SAS Version 8.

Analysis of the *E. nitens* half-diallel, the *E. globulus* factorial and *E. nitens x globulus* factorial to obtain variance components and genetic parameters at each test temperature were carried out according to the following model (in matrix notation):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Ws} + \mathbf{e} \quad \text{(Equation 3.4)}$$

where **y** is a n x 1 vector of the mean relative conductivity (RC) for each seedling, **b** is a p x 1 vector of the overall mean, **a** is a q x 1 vector of unobservable additive genetic effects of the seedlings and parents, and in the case of *E. globulus* and the F_1 hybrids the genetic group (parental provenance -

T, K, F), **s** is a r x 1 vector of genetic effects common to a full-sib family *i.e.* specific combining ability (SCA) effects equivalent to one quarter of the dominance effects (Falconer and MacKay 1996) assumed random, and **e** is a n x 1 vector of residuals which includes three quarters of the dominance and environmental error. **X**, **Z**, and **W** are known incidence matrices relating observations **y** to effects in **b**, **a** and **s** respectively. The model assumes all random terms are multivariate normally distributed random variables with zero means and (co)variances as follows:

$$\mathbf{Var}\begin{bmatrix}\mathbf{y}\\ \mathbf{a}\\ s\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\mathbf{ZGZ'+R} & \mathbf{ZG'} & \mathbf{WD'} & \mathbf{R}\\ \mathbf{GZ'} & \mathbf{G} & \mathbf{0} & \mathbf{0}\\ \mathbf{DW'} & \mathbf{0} & \mathbf{S} & \mathbf{0}\\ \mathbf{R} & \mathbf{0} & \mathbf{0} & \mathbf{R}\end{bmatrix} \qquad \text{(Equation 3.5)}$$

where $\mathbf{G} = \text{Diag}\{\mathbf{A} \times \mathbf{T_A}; \mathbf{I} \times \mathbf{T_S}\}$ is the genetic variance-covariance matrix, **A** the numerator relationship matrix between trees, $\mathbf{T_A}$ the matrix of additive genetic covariance of traits, and $\mathbf{T_S}$ the matrix of covariances for the additional random effects (Meyer 1989), **R** is the matrix of variance-covariance of the residuals and **S** the matrix of variance-covariance of the SCA effects.

Estimates of variance components were obtained by restricted maximum likelihood (REML) procedures (Patterson and Thompson 1971). REML is an iterative procedure where each iteration comprises an evaluation of the likelihood for a given set of variance components. The process is repeated until the likelihood is maximized. Assuming normality, an expression for this likelihood (**L**), or equivalently, -2 times its logarithm, is:

$$-2\log\mathbf{L} = \text{const} + \log|\mathbf{R}| + \log|\mathbf{G}| + \log|\mathbf{C}| + \mathbf{y'Py} \qquad \text{(Equation 3.6)}$$

where **R** and **G** are as defined above, and **C** is the coefficient matrix in Henderson's Mixed Model Equations (Henderson 1975). The matrix **P** is called the projection matrix and is given by:

$$\mathbf{P} = \mathbf{V}^{-1} - \mathbf{V}^{-1}\mathbf{X}(\mathbf{X}'\mathbf{V}^{-1}\mathbf{X})^{-}\mathbf{X}'\mathbf{V}^{-1} \qquad \text{(Equation 3.7)}$$

where $\mathbf{V} = (\mathbf{R} + \mathbf{ZGZ}' + \mathbf{WSW}')$ is the total variance. The maximum of **L** (or the minimum of -2log**L**) and the derivation of variances and covariances were carried out using the program ASREML (Gilmour *et al.* 1999).

For CP material, estimates of additive, SCA and error variances (σ^2_a, σ^2_{sca} and σ^2_e respectively) were used to estimate narrow sense heritabilities (h^2_{CP}) as:

$$h^2_{CP} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{sca} + \sigma^2_e} \qquad \text{(Equation 3.8)}$$

For OP progeny (GOP, GSOP, and NOP), a family model was fitted with female provenance as a fixed effect. In this case, additive genetic variance (σ^2_a) was estimated as: $\sigma^2_a = (1/r)\sigma^2_{fam(ctype)}$, where r is the coefficient of relatedness, assumed to be 0.4, which attempts to adjust for an outcrossing rate of 70% and is a common assumption in analyses of OP eucalypt families (Griffin and Cotterill 1988; Volker *et al.* 1990; Potts and Jordan 1994a). The heritability was then calculated as:

$$h^2_{OP} = \frac{\sigma^2_a}{(\sigma^2_{fam(ctype)} + \sigma^2_r)} \qquad \text{(Equation 3.9)}$$

where $\sigma^2_{fam(ctype)}$ is the among family within cross-type variance and σ^2_r is the residual variance in this analysis.

For CP progeny, the dominance ratio, d^2, an estimate of the relative significance of dominance assuming no higher order gene interactions such as

epistasis (Becker 1984), was calculated from the variances estimated from the univariate individual tree model as:

$$d^2 = \frac{4\sigma^2_{sca}}{\sigma^2_a + \sigma^2_{sca} + \sigma^2_e} \qquad \text{(Equation 3.10)}$$

Sampling errors of these estimates are usually approximated by the inverse of the matrix of second derivatives (also called the Hessian matrix) of the log likelihood function with respect to the parameters to be estimated. However a Hessian matrix is not available with ASREML algorithms and an approximation was obtained by numerical differentiation as described by Nelder and Mead (1965). An individual tree, multivariate model was fitted to estimate the genetic correlations between traits in different cross types (*e.g.* GCP vs GOP & GSOP; GCP vs F_1 vs NCP and NCP vs NOP). The genetic correlations and their standard errors were estimated from the relevant variance and covariance components using the CORR function in ASREML. This uses a Taylor series approximation to derive standard errors (Gilmour *et al.* 1999). Parameter estimates that differed by more than 2 standard errors from zero were treated as significant (Gilmour *et al.* 1999).

Results and Discussion

Means

Frost tolerance, as measured by the relative conductivity (RC), was clearly superior in *E. nitens* compared with *E. globulus*. Relative conductivity in *E. nitens* control-pollinated progeny (NCP) decreased slightly, from around 0.88 at -5.5°C to around 0.83 at -8.5°C (Table 3.2). In *E. globulus* control-pollinated progeny (GCP) the drop in RC with decreasing temperatures was more marked, from around 0.86 at -5.5°C to 0.61 at -8.5°C (Table 3.2). The

differences in RC between *E. nitens* and *E. globulus* were not significant at -5.5°C, but became larger and statistically significant at -8.5°C ($P < 0.001$) (Table 3.2).

Differences among *E. globulus* provenances (TT and KK) and inter-provenance crosses (intra-specific hybrids - TK, KT, FK, FT) were also apparent at all temperatures (Table 3.2; Fig. 3.2). The Taranna provenance (TT) was statistically more frost resistant ($P<0.05$) than King Island (KK) at temperatures -7.0°C and -8.5°C, with their inter-provenance crosses (TK and KT) being intermediate (Table 3.2; Fig. 3.2) and not statistically different from either parental provenance. Crosses between Flinders Island and either Taranna (FT) or King Island (FK) were not significantly different from each other and were also intermediate between TT and KK (Table 3.2). *E. globulus,* OP progeny (GSOP and GOP combined) were less tolerant to frost than CP progeny (GCP) for Taranna provenance at all temperatures ($P<0.0001$) whereas for King Island the OP progeny were only less tolerant at -7.0 °C ($P<0.01$) (Table 3.2). OP families from Taranna provenance were more frost sensitive ($P<0.05$) than from KI provenance at -5.5°C and -7.0°C only. In *E. nitens* there was no significant difference in RC between the control-pollinated (NCP) and the open-pollinated (NOP) progeny at any temperature (Table 3.2; Fig. 3.1). The apparent poorer performance of open-pollinated material to frost found in *E. globulus* has also been found in other traits (Hodge *et al.* 1996 and Chapter 4 in this thesis) and suggests that inbreeding depression occurs in the progeny as a result of selfing and related mating among closely related neighbours. Such neighbourhood inbreeding is likely to be more frequent in native stands (Eldridge *et al.* 1993; Hardner *et al.* 1996) than the seed orchard, which is consistent with the slightly poorer performance of GOP than GSOP seedlots.

Table 3.2 Arithmetic means (mean) and standard errors (s.e.) based on family averages of RC for different cross types of *E. globulus, E. nitens* and *E. nitens x globulus* at three test temperatures. N is the number of families in each cross type. Overall mean for each grouped cross type is shown in bold. Provenance is indicated in *E. globulus* and *E. nitens x globulus* as T = Taranna, K = King Island, F = Flinders Island, in *E. nitens* and *E. nitens x globulus* as N = Toorongo. GCP = *E. globulus* control-pollinated, GSOP = *E. globulus* open-pollinated from seed orchard, GOP = *E. globulus* open-pollinated from native stands, F_1 Hybrid = *E. nitens x globulus*, NCP = *E. nitens* control-pollinated, NOP – *E. nitens* open-pollinated.

Species	Cross type	N	Test temperature					
			-5.5°C		-7.0°C		-8.5°C	
			mean	**s.e.**	**mean**	**s.e.**	**mean**	**s.e.**
E. globulus	TT	19	0.872	0.005	0.831	0.007	0.640	0.019
	TK, KT	20	0.864	0.003	0.803	0.005	0.596	0.014
	KK	11	0.856	0.004	0.798	0.010	0.582	0.019
	FT	4	0.853	0.013	0.817	0.013	0.594	0.042
	FK	4	0.858	0.006	0.812	0.012	0.615	0.023
	GCP	**58**	**0.864**	**0.005**	**0.813**	**0.008**	**0.609**	**0.015**
	T - SOP	4	0.836	0.010	0.750	0.015	0.470	0.023
	K - SOP	3	0.840	0.009	0.743	0.043	0.522	0.048
	F - SOP	1	0.847	-	0.792	-	0.564	-
	GSOP	**8**	**0.839**	**0.010**	**0.752**	**0.025**	**0.501**	**0.031**
	T - OP	10	0.808	0.024	0.691	0.032	0.456	0.020
	K - OP	4	0.839	0.023	0.734	0.039	0.504	0.045
	GOP	**14**	**0.817**	**0.024**	**0.703**	**0.035**	**0.470**	**0.033**
E. nitens x globulus	NT	28	0.867	0.003	0.801	0.006	0.554	0.015
	NK	12	0.876	0.008	0.827	0.009	0.611	0.027
	F_1 Hybrid	**40**	**0.870**	**0.005**	**0.808**	**0.007**	**0.571**	**0.020**
E. nitens	NCP	34	0.880	0.003	0.869	0.003	0.829	0.006
	NOP	9	0.900	0.003	0.884	0.006	0.817	0.016

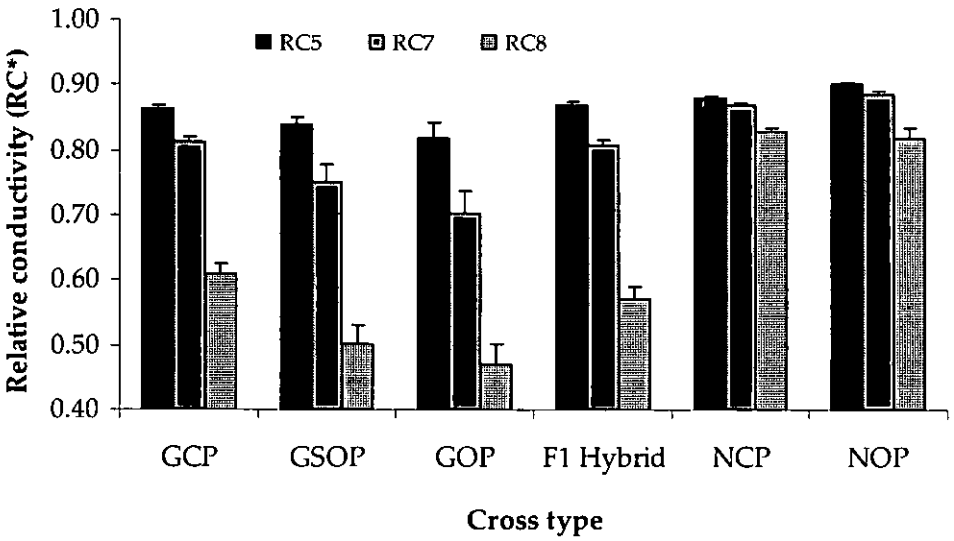


Figure 3.1 Mean relative conductivity (RC) at three test temperatures -5.5C (RC5). -7.0C (RC7), -8.5C (RC8) of families of *E. globulus* control-pollinated (GCP), open-pollinated from seed orchards (GSOP), open-pollinated from natural stands (GOP), *E. nitens x globulus* F_1 Hybrids (F1 Hybrid), *E. nitens* control-pollinated (NCP) and open-pollinated (NOP). Standard error bars are shown above each column.

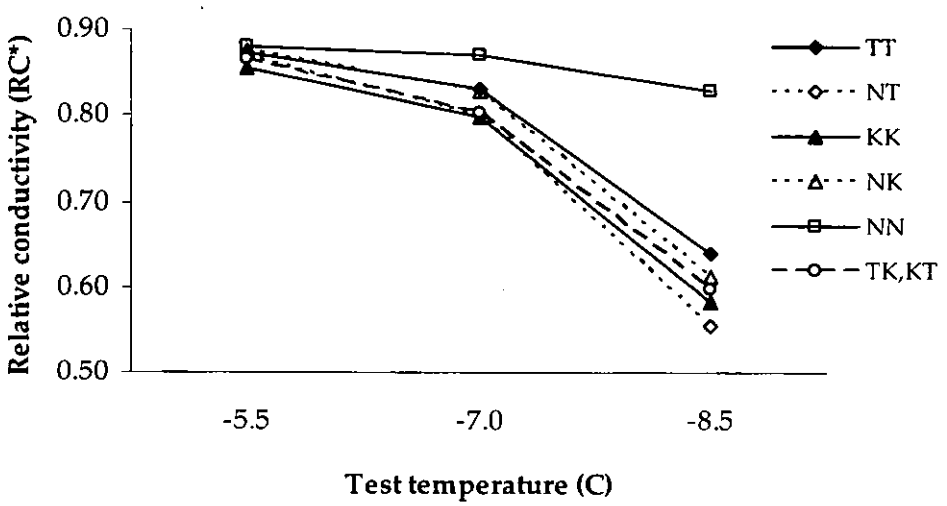


Figure 3.2 Relative conductivity (RC) at three test temperatures of intra-specific hybrid in *E. globulus*(TK,KT) and interspecific F1 hybrid of *E. nitens x globulus* (NT and NK) and pure species crosses in *E. globulus* (TT and KK) and *E. nitens* (NN).

Raymond *et al.* (1992a) found the value of RC = 0.8 as the limit between reversible and irreversible damage in *E. nitens* and *E. regnans* leaf tissue with values lower than 0.8 indicating irreversible damage. Furthermore, the mean RC value for discs taken from leaves was shown to be strongly correlated with the amount of leaf damage in seedlings, with 0.8 corresponding to 50% leaf damage (Raymond *et al.* 1992a). Although the relationship has not been established for *E. globulus* it is reasonable to assume that it would be similar. The RC values for *E. nitens* suggest that the species would suffer little damage even at the lowest temperature of -8.5°C and much lower temperatures could have been tolerated before a 50% damage (or RC = 0.8) is reached. In *E. globulus* it can be determined that RC = 0.8 is reached at about -7.0°C in GCP progeny and at slightly higher temperatures in GOP and GSOP. Although the correspondence between bath temperatures and natural frost temperatures are not well established, these critical temperatures agree well with direct estimates of frost tolerance in hardened *E. globulus* seedlings in Portugal (Almeida *et al.* 1995).

In the inter-specific F_1 hybrid those crosses involving King Island *E. globulus* (NK) only showed significant negative mid-parent heterosis at -8.5°C, whereas the F_1's with Taranna *E. globulus* (NT) show significant negative mid-parent heterosis which is larger in magnitude at -7.0°C and -8.5°C (Table 3.3) and in both cases these inter-specific hybrids are not significantly different from intra-specific crosses in *E. globulus.* The intra-specific hybrid (TK, KT) in *E. globulus* is not significantly different from the mid-parent at all temperatures (Table 3.3). In both cases the inter-specific hybrid is not different in frost tolerance to the *E. globulus* CP (Table 3.2, Fig. 3.2). The tendency of the F_1 *E. nitens x globulus* to be closer in performance to the less sensitive *E. globulus* has previously been observed (Tibbits *et al.* 1991b).

Table 3.3: Heterosis estimates (%) for mean RC in intra-specific (*E. globulus*) and interspecific hybrids (*E. nitens x globulus*) at three test temperatures. T = Taranna, K = King Island in *E. globulus* and N = Toorongo *E. nitens*. TK is compared against the mid-parent of TT with KK, NT and NK are compared against the mid-parent of TT with NN and KK with NN respectively. Tests of significance ^{ns} – not significant, * P<0.05, ** P<0.001, ***P<0.0001.

Hybrid class		Cross type	Test temperature		
			-5.5°C	-7.0°C	-8.5°C
Intra-specific hybrid	*E. globulus*	TK, KT	0.0^{ns}	-1.4^{ns}	-2.5^{ns}
Inter-specific hybrid	*E. nitens x globulus*	NT	-1.0^{ns}	-5.8***	-24.6***
		NK	0.9^{ns}	-0.8^{ns}	-13.4**

Variances and covariances

In *E. globulus* the GCP additive (σ^2_a), SCA (σ^2_{sca}) and error (σ^2_e) variances generally increase with decreasing freezing temperature (Table 3.4). This was associated with an overall increase in the level of damage with lower temperatures. As damage approaches the RC = 0.8 level at -7.0°C the heritability is not significantly different from zero. At -8.5°C, where there was a significant level of damage, with most material killed (*i.e.* RC < 0.7) variances were greatly inflated compared with lower temperatures and heritability increased to 0.46 (Table 3.4).

Estimates of OP additive and error variance (GOP, GSOP, NOP) were obtained from a family model, as it was not possible to fit an individual tree model. The true error variance in these cases was not significantly different from zero (data not shown), indicating the lack of replication of family information in the trial (a maximum of 10 individuals per OP family). Whereas in CP, an individual tree model was fitted and although there were

only 10 trees per family tested, each male and female was represented across a number of families.

Variance components also change with temperatures in open-pollinated progeny and were much greater than corresponding variances in CP (Table 3.4). In GSOP, variances increased from -5.5°C to -8.5°C, reflecting increased levels of damage. The corresponding heritability estimates had very large standard errors. The heritability estimates appear to be similar in magnitude for GSOP and GCP at -5.5 °C, where there was little damage. In contrast GSOP heritability estimates were greater than GCP estimates at the lower temperatures. In GOP, the variances peaked at -7.0°C with heritability estimates not significantly different from one at all temperatures. The most likely explanation for the drop in variance at -8.5°C is that most of the material was killed. Extrapolating from calculations of Raymond *et al.* (1992a), the RC value of 0.47 (Table 3.2) would correspond to 90 to 100% mortality for the GOP progeny at -8.5°C.

In *E. nitens* CP, there was little change in the phenotypic variance ($= \sigma^2_a + \sigma^2_{sca} + \sigma^2_e$) between RC5 and RC8 (0.90 and 0.76 respectively), reflecting the lack of damage caused by these test temperatures. Heritabilities were high but with very high standard errors (Table 3.4). *E. nitens* OP gave larger error variances and inflated heritabilities compared with CP, but also with very high standard errors (Table 3.4).

In the *E. nitens x globulus* F_1 estimates of the variance components are similar in magnitude at all temperatures to those found in *E. globulus* CP (Table 3.4) and appear to behave in the same way, which is reflected in the similarities for h^2 at all temperatures, although in both cases the heritability is only significantly different from zero at -5.5°C.

Table 3.4 Additive (σ^2_a), SCA (σ^2_{sca}) and error variance (σ^2_e), narrow sense heritability (h^2) and approximate standard errors, proportion of dominance (d^2) for each cross type within species and test temperature, for *E. globulus, E. nitens* and *E. nitens x globulus*. In the case of the OP families, the error variance presented is directly from the family model, includes a component of the additive variation $1.5*\sigma^2_{fam(ctype)}$ and represents the variation within OP families. The error variance estimate from the individual tree analysis of CP families does not include an additive genetic component.

Species	Cross type	Test temp.	σ^2_a (x 10^{-3})	σ^2_{sca} (x 10^{-3})	σ^2_e (x 10^{-3})	h^2	s.e.	d^2	s.e.
E .globulus	**GCP**	**-5.5**	.27	.15	.29	.38	(.18)	.82	(.24)
		-7.0	.23	.57	1.62	.10	(.13)	.94	(.25)
		-8.5	6.00	2.45	4.49	.46	(.21)	.78	(.26)
	GOP	**-5.5**	6.19	-	6.05	1.02	(.30)	-	-
		-7.0	16.68	-	14.70	1.13	(.30)	-	-
		-8.5	11.06	-	8.87	1.25	(.30)	-	-
	GSOP	**-5.5**	.56	-	1.37	.41	(.34)	-	-
		-7.0	6.19	-	6.11	1.01	(.45)	-	-
		-8.5	7.90	-	12.57	.63	(.40)	-	-
E. nitens	**NCP**	**-5.5**	.51	.25	.14	.57	(.57)	1.10	(1.81)
		-7.0	.24	.23	.29	.31	(.25)	1.21	(.42)
		-8.5	1.92	.47	.51	.66	(.43)	.65	(1.08)
	NOP	**-5.5**	.14	-	.40	.34	(.25)	-	-
		-7.0	.69	-	.68	1.02	(.36)	-	-
		-8.5	5.29	-	5.06	1.05	(.36)	-	-
E. nitens x globulus	**F_1 Hybrid**	**-5.5**	.43	.09	.30	.53	(.20)	.42	(.22)
		-7.0	.65	.47	1.72	.23	(.16)	.66	(.25)
		-8.5	4.85	3.89	6.55	.32	(.20)	1.02	(.33)

Dominance effects were large for *E. globulus, E. nitens* and the F_1 hybrid, accounting for 42 to 100% of the total variance (Table 3.4), but with very high standard errors. While there is evidence that dominance variance decreases over time for growth traits, even at early ages the levels reported in Chapter 5 for growth traits are lower than found here for frost traits. SCA effects in this experiment are likely to be inflated by the trial design and sampling strategy where families were not replicated in the nursery and all seedlings in a family were sampled at one time. However, while little environmental variation was detected across the nursery in the control families tested, the SCA estimates

are likely to confound true SCA effects and environmental differences between families. This high estimated SCA effect could be a major contributor to the inflated h^2 estimate in the OP material.

Table 3.5 Genetic correlation (r_g), with standard errors in parentheses, of RC for CP with OP and interspecific F_1 hybrids in *E. globulus* and *E. nitens* at three test temperatures

Species	Cross type	Test temperature	OP	F_1 hybrid
E. globulus	CP	-5.5 °C	-0.60 (0.34)	0.04 (0.62)
		-7.0 °C	-0.14 (0.58)	-0.26 (0.87)
		-8.5 °C	0.80 (0.40)	-0.25 (0.61)
E. nitens	CP	-5.5 °C	0.77 (0.71)	-0.66 (0.43)
		-7.0 °C	0.40 (0.31)	-0.31 (0.82)
		-8.5 °C	0.80 (0.34)	-1.08 (0.21)

The low genetic correlation of CP with OP performance in *E. globulus* (Table 3.5) was not significantly different from zero at -5.5°C and -7.0°C and it was only at the lowest temperature of -8.5°C that the correlation was positive and significantly different from zero. At this temperature there was considerable damage to the *E. globulus* CP and the h^2 was the highest of all temperatures (Table 3.4). In contrast within *E. nitens* there were reasonably strong genetic correlations between OP and CP performance at all temperatures, although standard errors were high and the correlation was only significant at -8.5°C (Table 3.5).

The results presented here initially indicate that, for the frost traits examined, the F_1 inter-specific hybrid demonstrates similar characteristics to the *E. globulus* parent at the population level. However, within cross types the genetic correlation (r_g) of pure species with inter-specific F_1 hybrid

performance (*i.e.* correlation of GCA with GHA) was not significantly different from zero in *E. globulus* (around r_g = -0.25 at -7.0°C and -8.5°C; Table 3.5). In *E. nitens* there was a high negative genetic correlation of r_g = -1.08±0.21 at -8.5°C, while correlations at other temperatures were not significantly different from zero (Table 3.5). This single significant result could arise if different genes are the determinants of the trait in hybrid population compared to one or both of the parental populations (Vigneron and Bouvet 2000), the hybrid combinations are influenced more by non-additive than additive effects (Dieters and Dungey 2000) or other genetic factors such as chromosomal structural differences impact on hybrid performance. This is further discussed in Chapter 5.

Conclusion

Reliable estimates of genetic parameters of economically important traits are essential to the success of a breeding program. Risk of significant frost damage in *Eucalyptus* is known to vary due to environmental and physiological conditions pertaining at a particular locality and time (Eldridge 1968; Raymond *et al.* 1986; Tibbits and Reid 1987a; Hallam and Tibbits 1988; Tibbits and Hodge 2001). While it may not be possible to account for all these factors when determining the absolute levels of resistance, results suggest that provenances and individual trees can be accurately ranked when grown in a common environment and tested at the same time. Previous studies with *E. nitens* (Tibbits and Reid 1987a; Tibbits *et al.* 1991b; Raymond *et al.* 1992b; Tibbits and Hodge 2001) show a significant variation in frost tolerance, with the trait being under moderate to strong additive genetic control. In *E. globulus* CP, heritabilities were higher at RC5 and RC8 but low at RC7 which is not consistent with the premise that heritabilities are highest at RC values

around 0.7 - 0.8, (*i.e.* at intermediate levels of leaf damage), which has been demonstrated as a statistical property of the trait used (Raymond *et al.* 1992a).

Large dominance effects found in this study may have been confounded by the lack of replication of families in the field trial and sampling strategy employed. This is a limitation of the experimental design and the capability of the equipment, where material must be sampled over a number of days or weeks. However, this is unlikely to impact markedly on h^2_{CP} estimates or cross type effects as the families themselves were randomised in space and time.

It is clear from the results presented here, and previous studies in other species with other traits, that the use of OP material can result in lower frost tolerance levels and inaccurate estimates of additive genetic variance when compared with CP crosses from the same parent.

The present study suggests that, like growth traits (Chapter 5), frost tolerance of leaf tissue using the electro conductivity method also exhibits inbreeding depression in *E. globulus* and this is likely to affect breeding value estimates. The tolerance of OP seedlings is significantly less than that of CP progeny, and these OP progeny are likely to contain varying levels of inbreeding (Hardner and Potts 1995a). Furthermore, the correlation between breeding values estimated from OP and CP populations is poor and only significant at the most discriminating frost temperature. In contrast, there was no evidence of inbreeding depression for frost tolerance in the OP progenies of *E. nitens* and while still only significant at the lowest temperature, correlations between OP and CP performance were constantly positive. This result suggests that gene action is affected by cross type. OP estimates of genetic variances are unreliable for these traits especially where there may be inbreeding effects extant in native populations.

It is clear that frost resistance is inherited in different manner in the inter-specific F_1 hybrid compared to the inter-provenance hybrids of *E. globulus*. While the significant differences between TT and KK were inherited in an intermediate manner in the inter-provenance F_1, the inter-specific F_1 hybrid between *E. nitens* and *E. globulus* exhibits frost tolerance comparable to the less tolerant species, *E. globulus*. Furthermore, there is no correlation between parental performance in intra-specific crosses compared with inter-specific crosses (*i.e.* comparison of GCA with GHA) suggesting different genes or gene interactions are at work in the different cross types. This also appears to be more pronounced in the Taranna provenance of *E. globulus* than the King Island provenance. The lack of correlation between GCA and GHA means that performance in inter-specific F_1 hybrids cannot be predicted from performance in pure species crosses. The sample size in this experiment is small relative to the whole specific gene pools, which is reflected by high standard errors associated with these correlation estimates. Therefore these correlation estimates are somewhat unreliable and may differ with a larger sample size. The correlation of GCA and GHA is discussed further for other traits in Chapter 5.

It appears there is no overall advantage in a hybrid combination over pure *E. globulus* for the objective of improving frost resistance as was initially hoped.

Chapter 4: Comparison of genetic parameters for open-pollinated and control-pollinated families of *Eucalyptus globulus* and *E. nitens* grown across multiple sites

Introduction

Most *Eucalyptus* species are still in the early stages of domestication and the majority of breeding programs have been based on estimates of genetic parameters (*e.g.* narrow sense heritability, genetic correlations) derived from open-pollinated (OP) populations (Eldridge *et al.* 1993). The accuracy of such genetic parameters and predicted breeding values, however, has been questioned. Most eucalypt species have mixed mating systems (Potts and Wiltshire 1997) and simulation studies have shown that heterogeneous selfing rates and associated dominance effects can bias heritability estimates in OP (Borralho 1994). Resende *et al.* (1995) have shown that overestimates of genetic gain are always obtained when mixed mating systems are not taken into account. OP heritabilities are estimated assuming that variation among OP families mainly reflects additive genetic variation amongst the female parents. However, there is increasing evidence in *Eucalyptus* that variation in growth rate, at least, is associated with variation in outcrossing rates amongst the female parents. A comparison of selfs with OP and control-pollinated (CP) progeny in *E. regnans* demonstrated inflated heritability estimates for OP, while self and CP estimates were similar (Griffin and Cotterill 1988). In this case OP heritability estimates appeared to be inflated by heterogeneity in outcrossing rates and inbreeding depression which were also influenced by

mortality and stand age (Hardner and Potts 1995b). Burgess *et al.* (1996) have clearly demonstrated that variation in the growth performance of native stand OP progeny was associated with different outcrossing rates in *E. grandis*. Lower outcrossing rates in parental populations resulted in poorer mean growth rate of progeny. In the case of *E. globulus*, the growth of OP families was significantly affected by the density and isolation of the native stand from which the seed was collected (Borralho and Potts 1996), which appeared to be due to higher outcrossing in the more dense stands (Hardner *et al.* 1996). Subtle variation in outcrossing rate may markedly influence the average growth rate of OP progenies of *E. globulus* as inbreeding is severe. For example, selfs are at least 25% smaller in diameter at 4 years than control-pollinated (CP) progenies from the same parents (Hardner and Potts 1995a; Hardner *et al.* 1998).

Estimates of OP genetic parameters for *E. globulus* have generally been derived from provenance/progeny trials established using native forest sourced seed, in particular from the Orme collection (Orme 1977; Volker and Orme 1988) and the more comprehensive range-wide *E. globulus* ssp. *globulus* base population collection undertaken by CSIRO (Gardiner and Crawford 1987; 1988). Heritabilities have been reported from these trials for numerous traits including growth traits such as diameter, height, cross sectional area and volume (Volker *et al.* 1990; Ipinza *et al.* 1994; Potts and Jordan 1994b; Borralho *et al.* 1995; MacDonald *et al.* 1995; MacDonald *et al.* 1997), pulp yield and basic density (Dean *et al.* 1990), leaf morphology (Potts and Jordan 1994a), flowering time (Gore and Potts 1995), drought tolerance (Dutkowski 1995), survival (Chambers *et al.* 1996), flowering precocity (Chambers *et al.* 1997), wood density (MacDonald *et al.* 1997), timing of vegetative phase change and first flowering (Jordan *et al.* 1999). Genetic parameters for growth and wood

quality of OP *E. globulus* material derived from other sources have also been reported (Woolaston *et al.* 1991a; 1992b; Borralho *et al.* 1992a; 1992c).

Two major paper companies commenced domestication of *E. nitens* in Australia in the early 1980's (Australian Paper Manufacturers in Gippsland, Victoria and Associated Pulp and Paper Mills in Tasmania). The State forest services in Victoria and Tasmania also commenced work but at a lower intensity. Genetic parameter estimates using progeny of *E. nitens* OP material collected in native stands, have been reported for growth (Purnell 1986; Woolaston *et al.* 1991b; Whiteman *et al.* 1992; Kube *et al.* 1996; Gea *et al.* 1997; Tibbits and Hodge 1998), frost tolerance (Tibbits and Reid 1987a; Raymond *et al.* 1992b; Tibbits and Hodge 2001), Pilodyn (Greaves *et al.* 1995), basic density and pulp yield (Greaves *et al.* 1995; Gea *et al.* 1997; Greaves *et al.* 1997b; Tibbits and Hodge 1998).

In 1986 a control-pollination program within and between *E. globulus* and *E. nitens* was commenced (see Chapter 2). One of the objectives of the crossing program was to determine if genetic parameters estimated from open-pollinated material were reliable. The only way to empirically test this hypothesis was to undertake control-pollination with the same parental material from which OP seed was sampled and directly compare genetic parameters estimated from both CP and OP material. The program was also used to determine the relative importance of additive and non-additive genetic effects, which cannot be determined using OP progeny.

Genetic parameters from these crosses have previously been reported for frost tolerance (Volker *et al.* 1994), growth (Hodge *et al.* 1996), *Mycosphaerella* leaf disease (Dungey *et al.* 1997), timing of vegetative phase change and first flowering (Jordan *et al.* 1999). Chapter 5 in this thesis also compares genetic parameters for growth and Pilodyn penetration of within and between

provenance crosses in *E. globulus*, within provenance crosses in *E. nitens* and *E. nitens x globulus* F_1 hybrids using the same parents at one site.

The present chapter directly compares genetic parameters and the performance of progeny derived from the same parents, used in OP and CP, for both *E. globulus* and *E. nitens*. The traits examined are growth (as estimated by diameter at various ages up to 6 years) and Pilodyn penetration at age 6 years, an indirect measure of wood density (Raymond *et al.* 1998) at individual sites. An across site analysis is also used to compare the effect of age on genetic parameter estimation and the magnitude of genotype by environment interaction, for OP and CP material.

Materials and methods

Crossing design

The crossing design used in this study is described in Chapter 2 (Table 2.1). This chapter relates to comparison of control-pollinated (CP) and open-pollinated (OP) material within *E. globulus* and *E. nitens*. The notation used for identifying cross types is GCP (*E. globulus* CP), GOP (*E. globulus* native stand OP), GSOP (*E. globulus* seed orchard OP), NCP (*E. nitens* CP) and NOP (*E. nitens* OP). In the genetic parameter analysis of OP in *E. globulus*, seed orchard open-pollinated (GSOP; 8 parents from 3 provenances, 3 King Island, 1 South Flinders Island and 4 Taranna) and native stand open-pollinated (GOP; 26 parents from 2 provenances, 16 Taranna and 10 King Island) cross types were combined, after initial analyses showed there was little further information to be gained by treating them as separate cross types.

Field trials

The field trials are described in Chapter 2 and specific field trials used for this chapter are detailed in Table 4.1.

The survival at each site was variable. Severe mortality of *E. globulus* during the first year at the HA site was caused by frost. The FR site was influenced by mammalian browsing in the first two years. The other sites also were influenced by varying levels of defoliation, particularly by *Mycosphaerella* spp. leaf fungi (Dungey *et al.* 1997). Despite these problems six sites were able to provide sufficient data for analysis (WR, FL, FR, HA, PA and WA).

Measurements

The results reported here are the result of measurements of diameter at breast height (1.3 m) over bark which were taken on all trees in each trial in 1992 (D2), 1993 (D3), 1994 (D4) and 1996 (D6). WA was only measured for D3 and D6; FL was not measured for D3 and HA was not measured for D4. In 1996, a Pilodyn reading was made on three trees in each plot (P6) at all sites except HA. The Pilodyn measurement was an average of two readings taken on the western side of each tree according to the method described in Raymond and MacDonald (1998).

Table 4.1 Location and design of field trials for comparison of control-pollinated (CP) and open-pollinated (OP) families of *E. globulus* and *E. nitens*. The trials at West Ridgley, Flynn and Manjimup also included families and seedlots of other material not reported here.

Code	Site	State	Latitude	Longitude	Altitude (m)	Trial Design				Number of families in each trial			
						Replicates	Incomplete blocks per replicate	Plots per block	Trees per plot	*E. globulus*		*E. nitens*	
										CP	OP	CP	OP
WR	West Ridgley	TAS	41°09′S	145°46′E	160	4	15	20	5	168	32	35	9
FR	Franklin	TAS	43°04′S	146°53′E	370	4	9	13	5	73	26	9	6
HA	Hampshire	TAS	41°15′S	145°45′E	470	4	7	9	5	0	30	16	8
PA	Parkham	TAS	41°26′S	146°37′E	200	4	13	14	5	135	30	1	8
FL	Flynn	VIC	38°18′S	146°40′E	170	4	11	18	5	111	28	32	9
WA	Manjimup	WA	34°12′S	116°01′E	240	7	11	11	3	96	28	0	5

Data analysis

All trees which survived to age 6 were included in the analysis of growth regardless of their size. Quantitative genetic analyses were undertaken using the program ASREML, which uses the average information algorithm and sparse matrix technology (Gilmour *et al.* 1999) to calculate restricted maximum likelihood variances and covariances for the random effects in univariate and multivariate mixed models.

In general, variance and covariance components for CP progeny were calculated with an individual tree model as:

$$y = X_1 c + X_2 r + Z_1 a + Z_2 s + Z_3 b + Z_4 p + e \qquad \text{(Equation 4.1)}$$

where y is an n x 1 vector of individual diameter or mean Pilodyn measurements, c is a vector of fixed cross-type effects (*i.e.* provenance effects in *E. globulus* – TT, KK, [TK and KT], FT, FK), r is a vector of fixed site and replicate effects combined (in the case of single site analyses only the replicate effects from the site were used), a is a vector of additive genetic effects (i.e., breeding values of individuals and parents), s is a s x 1 vector of random genetic effects common to each full-sib family (i.e., specific combining ability), b is a b x 1 vector of random effects common to each incomplete block (within each replicate), p is a p x 1 vector of random effects common to each plot (*i.e.* plot effect), and e is an n x 1 vector of residuals, expected to include the remaining three quarters of the dominance variance and environmental effects. $\boldsymbol{X}_1$, $\boldsymbol{X}_2$, $\boldsymbol{Z}_1$, $\boldsymbol{Z}_2$, $\boldsymbol{Z}_3$ and $\boldsymbol{Z}_4$ are incidence matrices relating observations in y to effects in c, r, a, s, b and p respectively. OP progeny were examined using Equation 4.1, excluding the full-sib family term ($\boldsymbol{Z}_2 s$). It was the female provenance which was used as the vector of c in this case. The same general model was used for univariate and bivariate analyses. The term $\boldsymbol{X}_1 c$ was

excluded from the model for analysis of *E. nitens* CP and OP progeny as only one provenance was represented.

The analyses undertaken with the general model described above were of five types. The first type used only the CP data and fitted an individual tree model with a matrix of relationships derived from the pedigree of each tree used to estimate additive variances (Gilmour *et al.* 1999). The second type of analysis, used only CP data and followed a bivariate individual tree model with the same effects (minus the generally insignificant plot term) as the univariate model for each trait and unconstrained covariances between traits. This model was used to determine the correlation between traits within a cross type. The third type used a univariate individual tree model to estimate the levels of additive variation among females from the OP cross types. The fourth type was the bivariate extension of the previous analysis used to estimate genetic correlations between traits of the OP progeny, but again with the plot term excluded. The fifth type used a bivariate, individual tree model of all data to estimate genetic covariances existing between CP and OP populations, from which genetic correlations were calculated. In this analysis the full sib family term Z_2s was only fitted for the CP population. The genetic correlations could be calculated due to the common parent pedigree links between these two populations.

The variance and covariance components estimated from these models were treated as significantly different from zero ($P < 0.05$) if the parameter estimate was more than twice the magnitude of the standard error, and not significant if the ratio was less than one (Gilmour *et al.* 1999). If the ratio was between 1 and 2 the significance of the estimate was tested using a likelihood ratio test (Gilmour *et al.* 1999). Tests of variances in the univariate analyses compared models including and excluding the relevant effect. Specific tests of the significance of the difference of correlations from zero were made by

comparing the likelihood ratio derived from the unconstrained bivariate model to that from a bivariate model where the correlation was constrained to zero.

Individual narrow sense heritabilities for CP were calculated as:

$$h^2_{CP} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{sca} + \sigma^2_{plot} + \sigma^2_e} \qquad \text{(Equation 4.2)}$$

where σ^2_a, σ^2_{sca}, σ^2_{plot} and σ^2_e are the additive within cross type, SCA within cross type, plot and residual variances from the univariate individual tree model respectively. These were compared with individual narrow-sense heritabilities from the OP progenies calculated as:

$$h^2_{OP} = \frac{0.625 \times \sigma^2_a}{(\sigma^2_a + \sigma^2_{plot} + \sigma^2_e)} \qquad \text{(Equation 4.3)}$$

σ^2_a in the individual tree model is calculated assuming OP families are half-sib families where the coefficient of relatedness (r) is 0.25. The additive variance estimate from the individual tree model was multiplied by 0.625, to adjust the coefficient of relatedness in OP families to be 0.4, which is a common adjustment used to account for an outcrossing rate of 70% in eucalypts (Griffin and Cotterill 1988; Volker *et al.* 1990; Potts and Jordan 1994a). These OP heritability estimates assume that non-additive variances are small.

For CP, the dominance ratio, d^2, an estimate of the relative significance of dominance assuming no higher order gene interactions such as epistasis (Becker 1984), was calculated from the variances estimated from the univariate individual tree model as:

$$d^2 = \frac{4\sigma^2_{sca}}{\sigma^2_a + \sigma^2_{sca} + \sigma^2_{plot} + \sigma^2_r} \qquad \text{(Equation 4.7)}$$

Genetic correlations were calculated from variance and covariance components according to the general formula:

$$r_{s(1,2)} = \frac{\sigma_{s(1,2)}}{\sqrt{\sigma^2_{s(1)}\sigma^2_{s(2)}}} \qquad \text{(Equation 4.8)}$$

where $r_{s(1,2)}$ is the correlation between traits 1 and 2 at level s (*e.g.* the additive genetic effects), $\sigma_{s(1,2)}$ is the covariance between traits at that level and $\sigma^2_{s(1)}$ and $\sigma^2_{s(2)}$ are the variance components for each trait at that level. Correlations between growth traits at various ages and Pilodyn were calculated from the additive variances and covariances estimated with the bivariate analysis of CP and OP data separately. These correlations indicate the degree of additive genetic dependence of the traits. Correlations between OP and CP populations for a specific trait were estimated directly using variances and covariances derived from equation 4.1 as previously described. These genetic correlations are based on the genetic effects nested within cross type and indicate the degree to which additive genetic effects are reflected in the genetic variation amongst OP families.

The expression of genetic effects across sites was examined using the more complex individual tree model:

$$y = X_1c + X_2l + X_3cl + X_4r + Z_1b + Z_2a + Z_3s + Z_4fl + Z_5ml + Z_6sl + e \qquad \text{(Equation 4.9)}$$

where in addition to the terms defined for equation 4.1, l is a vector of fixed site effects, r is a vector of fixed replicate effects, cl is a vector of fixed cross type by site effects, fl, ml and sl are vectors of random female by site, male by site and family by site effects respectively. $\boldsymbol{X}_1$, $\boldsymbol{X}_2$, $\boldsymbol{X}_3$, $\boldsymbol{X}_4$, $\boldsymbol{Z}_1$, $\boldsymbol{Z}_2$, $\boldsymbol{Z}_3$, $\boldsymbol{Z}_4$, $\boldsymbol{Z}_5$, and $\boldsymbol{Z}_6$ are incidence matrices relating observations in y to effects in c, l, cl, r, b, a, s, fl, ml, and sl respectively. The plot term was not included in this model as it

was generally insignificant and its inclusion caused difficulties in model convergence. For the analyses of the OP progenies, the terms associated with *sl*, *ml* and *s* were excluded from the model.

Across site, individual narrow sense heritabilities for CP were calculated as:

$$h^2_{CP} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{sca} + \sigma^2_{f.l} + \sigma^2_{m.l} + \sigma^2_{sca.l} + \sigma^2_e} \qquad \text{(Equation 4.10)}$$

where σ^2_a, σ^2_{sca}, $\sigma^2_{sca.l}$, $\sigma^2_{f.l}$, $\sigma^2_{m.l}$ and σ^2_e are the additive within cross type, SCA within cross type, SCA by site, female by site, male by site and residual variances from the univariate individual tree model respectively. These were compared with individual narrow-sense heritabilities from the OP progenies calculated as:

$$h^2_{OP} = \frac{0.625 \times \sigma^2_a}{(\sigma^2_a + \sigma^2_{f.l} + \sigma^2_e)} \qquad \text{(Equation 4.11)}$$

following the logic associated with equation 4.3. The across site dominance ratio, d^2, was calculated using the numerator from equation 4.7 with the denominator of equation 4.10.

The genotype by environment interaction was measured using a type B genetic correlation, defined as the correlation of genotype performance across environments or g x e (Burdon 1977; White and Hodge 1989) from which the equation has been interpreted as:

$$r_b = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_{ge}} = \frac{r\sigma^2_a}{r\sigma^2_a + \left(\left(\sigma^2_{f.s} + \sigma^2_{m.s}\right)/2\right)} \qquad \text{(Equation 4.12)}$$

where σ^2_g is the genotypic variance, σ^2_{ge} is the genotype x environment variance, $\sigma^2_{f.s}$, $\sigma^2_{m.s}$ are the female and male by site variance components

respectively, and r = 0.25 for CP and 0.4 for OP. In the case of the OP $\sigma^2_{m.s}$ was equated to $\sigma^2_{f.s}$.

The correlation of specific combining effects in CP across environments was calculated as:

$$r_{sca} = \frac{\sigma^2_{sca}}{\sigma^2_{sca} + \sigma^2_{sca.s}} \qquad \text{(Equation 4.13)}$$

where $\sigma^2_{sca.s}$ is the variance of interaction of SCA and site.

For comparison of cross types GCP, GOP, GSOP, NCP and NOP least squares means were estimated using equation 4.1 for each site where these cross types were the levels in the vector *c*. Inbreeding depression (ID) in open pollinated progeny was calculated following Hodge *et al.* (1996) as the difference in least squares mean value for OP (either GOP or GSOP) and CP material expressed as a percentage of the CP (or outcross) mean for each species at each site:

$$ID(\%) = \left[\frac{CP - OP}{CP}\right] \times 100 \qquad \text{(Equation 4.14)}$$

Results

Within site analysis

The least squares means (Table 4.2) show a general trend for growth within *E. globulus* of GCP>GSOP>GOP across all sites and ages. Within *E. nitens* there was little difference in performance between NCP and NOP. By age 6, diameter growth of *E. nitens* was significantly greater than that of *E. globulus* at all Tasmanian sites (WR, FR, PA, HA). *E. globulus* was significantly better

than *E. nitens* at FL (Victoria) while there was no significant difference between the two species at WA (Western Australia).

Inbreeding depression was evident in GOP with levels varying from 5% to 13% for growth traits across all sites (Table 4.2). GSOP showed much lower levels of inbreeding depression for growth at about 1 or 2 %, except at FR where levels were similar to GOP at about 8%. *E. nitens* showed little or no inbreeding depression for growth, with no significant difference between the performance of NCP and NOP (Table 4.2). In Pilodyn traits there was little inbreeding depression evident for *E. globulus* or *E. nitens*, with only GSOP at FR showing a level of 7%, which was still not significantly different from GCP least squares mean and NOP showing a level of -8% (Table 4.2).

In *E. globulus*, h^2_{OP} for D6 (range 0.11 to 0.35; average 0.24) was virtually double h^2_{CP} (0.07 to 0.18; average 0.12) for all sites except PA (Table 4.3). The comparison of h^2_{CP} with d^2_{CP} in *E. globulus* shows that dominance effects (range 0.05 to 0.31; average 0.14) are similar in magnitude to additive effects for D6 at all sites, although both genetic effects are quite low, except at WA where d^2_{CP} is relatively high (0.31) and h^2_{CP} is moderate (0.18). h^2_{CP} was significantly different from zero at WR, PA and WA only. d^2_{CP} and h^2_{OP} were significantly different from zero at WR and WA only.

Table 4.2 Least squares means (LSM) for each cross type for each trait (D2, D3, D4 and D6 being diameter at ages 2,3, 4 and 6 years, and P6 being Pilodyn at 6 years) obtained using equation 4.1 and inbreeding depression (ID) for OP compared with CP at each site. Cross type notation is detailed in the text. The overall standard error or the difference in is indicated (Overall SED). Inbreeding depression for Pilodyn is considered to be positive with greater Pilodyn penetration in the OP compared with CP (*i.e.* increased Pilodyn penetration is a less desirable trait). ID was not calculated for NOP at PA as only one NCP family could be used for comparison.

Site	Cross type	D2		D3		D4		D6		P6	
		LSM (mm)	ID (%)	LSM (mm)	ID (%)	LSM (mm)	ID (%)	LSM (mm)	ID (%)	LSM (mm)	ID (%)
WR	GCP	73.0		117.0		138.4		175.4		13.28	
	GOP	69.0	5	110.6	5	130.2	6	161.5	8	13.24	0
	GSOP	73.7	-1	117.2	0	137.4	1	172.5	2	13.47	2
	NCP	77.4		126.7		151.5		191.8		13.62	
	NOP	78.1	-1	127.5	-1	151.8	0	189.1	1	13.94	0
FL	GCP	52.2				123.6		159.2		11.29	
	GOP	47.4	9			114.9	7	144.8	9	11.32	0
	GSOP	51.9	1			122.5	1	156.8	1	11.50	2
	NCP	26.3				94.5		138.2		12.29	
	NOP	27.6	-5			95.5	-1	139.5	-1	12.34	0
FR	GCP	27.4		65.2		87.1		126.9		12.75	
	GOP	23.8	13	59.4	9	79.8	8	113.5	11	12.47	-2
	GSOP	25.2	8	60.1	8	81.4	7	116.5	8	13.32	7
	NCP	27.5		67.3		95.4		144.5		14.26	
	NOP	23.3	15	60.6	10	87.3	8	136.7	5	13.16	-8
PA	GCP	44.9		88.6		118.3		143.9		11.68	
	GOP	41.3	8	83.9	5	111.8	6	130.7	9	11.75	1
	GSOP	44.5	1	88.6	0	118.2	0	144.3	0	11.91	1
	NCP	35.1		80.1		131.2		163.5			
	NOP	47.7	-	94.8	-	131.3	-	166.0	-	12.03	
HA	GOP	32.9		66.6				113.1			
	GSOP	37.6		72.6				112.7			
	NCP	44.0		97.7				186.7			
	NOP	44.8	-2	99.5	-2			179.7	4		
WA	GCP			98.4				157.8		12.90	
	GOP			93.6	5			142.6	10	13.30	3
	GSOP			98.8	0			154.9	2	12.85	0
	NOP			101.4				168.0		13.17	
Overall SED		4.5		7.0		8.1		12.1		0.72	

Table 4.3 Comparison of variance components and heritabilities in CP and OP *E. globulus* and *E. nitens* at each site for D6. Standard errors are in parentheses. Parameters could not be estimated for *E. nitens* OP at FL.

SITE	Control - pollinated						Open - pollinated			
	σ^2_a (s.e.)	σ^2_{sca} (s.e.)	σ^2_{plot} (s.e.)	σ^2_e (s.e.)	h^2_{CP} (s.e.)	d^2_{CP} (s.e.)	σ^2_a (s.e.)	σ^2_{plot} (s.e.)	σ^2_e (s.e.)	h^2_{OP} (s.e.)
	E. globulus									
WR	119 (41)	22.5 (10.2)	9.7 (15.1)	705 (32)	0.14 (0.05)	0.10 (0.05)	406 (170)	0	761 (130)	0.35 (0.13)
FL	65 (32)	10.7 (11.8)	65.7 (19.3)	729 (32)	0.07 (0.04)	0.05 (0.06)	226 (133)	0	1186 (128)	0.16 (0.09)
FR	107 (63)	37.3 (21.9)	25.0 (24.5)	727 (48)	0.12 (0.07)	0.17 (0.10)	284 (137)	0	723 (188)	0.24 (0.11)
PA	71 (34)	9.7 (10.2)	31.2 (17.9)	692 (33)	0.09 (0.04)	0.05 (0.05)	100 (81)	0	773 (122)	0.11 (0.08)
WA	179 (55)	78.2 (14.3)	10.2 (13.1)	738 (37)	0.18 (0.05)	0.31 (0.05)	404 (169)	0	526 (221)	0.34 (0.13)
	E. nitens									
WR	611 (307)	35 (27)	21 (36)	450 (161)	0.55 (0.20)	0.13 (0.10)	115 (166)	113 (89)	704 (226)	0.11 (0.16)
FL	158 (137)	0	170 (97)	1476 (131)	0.09 (0.07)	0	1795 (251)	298 (290)	1077 (151)	0.57 (0.05)
HA	530 (325)	0	79 (67)	533 (181)	0.46 (0.23)	0	1795 (251)	289 (290)	1077 (151)	0.57 (0.05)
FR	6.7 (152)	0	300 (165)	1349 (186)	0.02 (0.51)	0	0	66 (172)	1059 (160)	0
PA	-	-	-	-	-	-	264 (252)	0	286 (317)	0.37 (0.31)
WA	-	-	-	-	-	-	113 (237)	0	774 (228)	0.13 (0.26)

The WR, HA , FR and FL sites were the only sites where there were multiple CP *E. nitens* families (Table 4.1) to make an estimate of variance components and genetic parameters (Table 4.3). These remaining sites showed considerable differences in performance (Table 4.2) for D6. At FL there is considerably more error variance, little additive variance and no SCA variance which contrasts with results for *E. globulus* CP at this site where error variance was comparable with other sites and d^2 was moderate.

The *E. nitens* h^2_{OP} estimate at WR was much less than the h^2_{CP} estimate (0.11 and 0.55 respectively), whereas at FL *E. nitens* h^2_{CP} was 0.09, while h^2_{OP} was a very high at 0.57 (Table 4.3). At FL, where *E. nitens* has much poorer performance than *E. globulus* (Table 4.2), it appears that *E. nitens* h^2_{CP} has been significantly reduced in comparison to WR and HA, which are sites where *E. nitens* is the better species for D6. The FR site, which only contained a few E. nitens families, demonstrated no significant additive genetic effect in *E. nitens* OP and CP. At all sites, d^2_{CP} was not significantly different from zero for *E. nitens*.

Estimates of *E. globulus* h^2_{CP} for P6 (range 0.28 to 0.32; average 0.30) were considerably higher than for D6, with low levels of d^2_{CP} (0 to 0.11; average 0.06) (Table 4.4), indicating a high level of additive genetic control of the Pilodyn trait. As for D6, the *E. globulus* h^2_{OP} for P6 (range 0.46 to 0.60; average 0.54) was considerably higher than the comparable h^2_{CP} estimate obtained at the same site. Virtually all heritability estimates had standard errors which indicated they were significantly different from zero (except h^2_{OP} for P6 at FL), whereas d^2_{CP} was only significant at WA.

Table 4.4 Comparison of variance components and heritabilities in *E. globulus* and *E. nitens* at each site for P6. Standard errors are in parentheses.

SITE	Control - pollinated						Open - pollinated			
	σ^2_a	σ^2_{sca}	σ^2_{plot}	σ^2_e	h^2_{CP}	d^2_{CP}	σ^2_a	σ^2_{plot}	σ^2_e	h^2_{OP}
	E. globulus									
WR	0.68 (0.21)	0.03 (0.02)	0	1.49 (0.12)	0.31 (0.08)	0.06 (0.05)	1.96 (0.69)	-	1.29 (0.54)	0.60 (0.18)
FL	0.39 (0.14)	0.04 (0.03)	0.09 (0.04)	0.87 (0.09)	0.28 (0.09)	0.11 (0.08)	0.87 (0.37)	0.05 (0.09)	0.77 (0.24)	0.51 (0.26)
PA	0.69 (0.22)	0	0.42 (0.06)	1.06 (0.13)	0.32 (0.09)	0	1.02 (0.46)	0.07 (0.12)	0.51 (0.57)	0.46 (0.20)
WA	0.59 (0.12)	0.04 (0.01)	0.19 (0.02)	1.20 (0.07)	0.29 (0.05)	0.08 (0.02)	1.96 (0.74)	-	0.11 (0.94)	0.60 (0.18)
	E. nitens									
WR	0.29 (0.24)	0.10 (0.09)	0	1.79 (0.20)	0.13 (0.11)	0.18 (0.16)	0.50 (0.75)	0.81 (0.86)	0.98 (0.51)	0.22 (0.50)
FL	0.92 (0.52)	0.05 (0.08)	0.09 (0.12)	1.38 (0.29)	0.38 (0.13)	0.08 (0.13)	0	0	1.68 (0.26)	0

In *E. nitens* at WR there was a low h^2_{CP} (0.13) for P6, with a higher estimate for h^2_{OP} (0.22), however both estimates were not significantly different from zero. At FL the *E. nitens* h^2_{CP} for P6 was relatively high (0.38) and significant while h^2_{OP} was zero. HA was not assessed for this trait.

Pooled analysis across sites

The pooled analysis across sites (Table 4.5) in *E. globulus* shows that h^2_{CP} for growth traits remains low and fairly constant (range 0.06 to 0.10; average 0.08) for the four ages measured. In contrast h^2_{OP} is always higher and shows moderate levels which increase with age (range 0.10 to 0.22; average 0.16). There is consistently higher additive and error variance estimates for growth of OP compared with CP. The h^2_{CP} estimate for D6 across sites (0.06) is considerably lower than the average of 0.12 derived from the individual site analyses (Table 4.3), whereas the OP estimates are similar in both cases (0.22 for across-site and 0.23 for average of individual sites). In P6 h^2_{CP} and h^2_{OP} in across-site analysis (0.25 and 0.61 respectively in Table 4.5) are similar to the averages derived from individual site analyses (0.30 and 0.54 respectively, derived from Table 4.4). Dominance variation (d^2_{CP}) is insignificant for all growth traits in the across-site analysis. The average d^2_{CP} for D6 using individual site analysis is 0.14 (derived from Table 4.3) compared with 0.02 for the across-site analysis (Table 4.5). d^2_{CP} is small and significant at 0.05±0.02 for P6 in the across site analysis (Table 4.5) and is similar to the average of the individual sites (0.06) from Table 4.4.

In *E. globulus*, type B correlations (r_b) were much larger for OP (range 0.61 to 0.92; average 0.78) than CP (range 0.36 to 0.64; average 0.52) for all growth traits at all ages whereas for P6, they were high for both OP (0.88±0.07) and CP (0.84±0.06) (Table 4.5). This indicates that there is more genotype by

environment interaction in CP material for growth traits, while P6 is stable across sites regardless of cross type. Type B correlations for diameter growth in both CP and OP material increased markedly between age 2 and 3 years and stabilized thereafter, indicating genetic effects for growth tend to be more stable across sites after about 2 years. The average inter-site correlation of SCA effects (r_{sca}) was low and not significantly different from zero for all growth traits but was relatively high for P6 ($r_{sca} = 0.72 \pm 0.33$). It is apparent that SCA effects appear to be more site specific for D6, where significant effects were found at WR and WA only (Table 4.3) and have been reduced to virtually zero in the across site analysis (Table 4.5). However, SCA effects for P6 are relatively stable even though they are quite small at each of the sites (Table 4.4).

In contrast to *E. globulus*, the h^2 estimates for OP and CP *E. nitens* were comparable in across site pooled analyses for all growth traits and P6 (Table 4.6). In all cases these estimates for growth were moderate to high (0.12 to 0.32; average 0.24). There was no significant d^2 for any trait. The averages of individual site analyses for D6 are comparable with across site analysis for OP and CP heritabilities (0.37 and 0.28 respectively, derived from Table 4.3) and for CP heritability for P6 (0.26 derived from Table 4.4). The average of the OP heritabilities for P6 from the two individual site analyses (0.11 derived from Table 4.4) is considerably lower than obtained from the across site analysis (0.35 ± 0.19; Table 4.6) where all sites with *E. nitens* were included in the analysis.

Type B correlations in *E. nitens* were usually lower in OP than CP for growth traits, although the differences were not significant except at D6 (Table 4.6). In P6 the correlation was effectively one, indicating that the additive genetic effects were perfectly correlated across sites with no genotype by environment interaction. There was no significant correlation of SCA effects across sites

(Table 4.6) as there were no significant SCA effects at the individual sites (Table 4.4).

Table 4.5 Genetic parameters (± standard error) for growth traits and Pilodyn penetration derived from pooled analysis of *E. globulus* CP and OP across sites.

TRAIT	Cross type	σ^2_a (s.e.)	σ^2_{sca} (s.e.)	$\sigma^2_{sca.s}$ (s.e.)	$\sigma^2_{f.s}$ (s.e.)	$\sigma^2_{m.s}$ (s.e.)	σ^2_{srb} (s.e.)	σ^2_e (s.e.)	h^2	d^2	r_b	r_{sca}
D2	CP	8 (4)	1 (1)	4 (1)	4 (2)	3 (1)	4 (1)	110 (3)	0.06 (0.03)	0.02 (0.02)	0.36 (0.14)	0.18 (0.16)
	OP	16 (7)			4 (3)		9 (3)	133 (7)	0.10 (0.05)		0.61 (0.21)	
D3	CP	24 (8)	1 (1)	6 (2)	4 (2)	3 (1)	39 (6)	213 (6)	0.10 (0.03)	0.01 (0.02)	0.64 (0.13)	0.14 (0.17)
	OP	61 (22)			4 (5)		34 (10)	271 (13)	0.18 (0.06)		0.87 (0.16)	
D4	CP	32 (12)	2 (2)	9 (3)	6 (3)	5 (2)	30 (4)	341 (8)	0.08 (0.03)	0.02 (0.02)	0.60 (0.13)	0.16 (0.17)
	OP	85 (34)			12 (9)		22 (9)	460 (28)	0.15 (0.06)		0.74 (0.17)	
D6	CP	48 (18)	5 (3)	19 (5)	18 (8)	5 (3)	26 (5)	725 (14)	0.06 (0.02)	0.02 (0.02)	0.51 (0.14)	0.20 (0.14)
	OP	272 (90)			10 (13)		23 (17)	940 (64)	0.22 (0.07)		0.92 (0.10)	
P6	CP	.47 (.14)	.02 (.01)	.01 (.01)	.02 (.01)	.02 (.01)	.20 (.03)	1.31 (.08)	0.25 (0.07)	0.05 (0.02)	0.84 (0.06)	0.72 (0.33)
	OP	1.43 (.46)			.08 (.04)		.34 (.08)	.82 (.28)	0.61 (0.15)		0.88 (0.07)	

Table 4.6 Genetic parameters (± standard error) for growth traits and Pilodyn penetration derived from pooled analysis of *E. nitens* CP and OP across sites.

TRAIT	Cross type	σ^2_a (s.e.)	σ^2_{sca} (s.e.)	$\sigma^2_{sca.s}$ (s.e.)	$\sigma^2_{f.s}$ (s.e.)	$\sigma^2_{m.s}$ (s.e.)	σ^2_{srb} (s.e.)	σ^2_e (s.e.)	h^2	d^2	r_b	r_{sca}
D2	CP	16 (10)	0	4 (3)	1 (2)	3 (3)	23 (5)	112 (7)	0.12 (0.07)	0	0.67 (0.26)	0
	OP	27 (22)			10 (7)		44 (11)	130 (16)	0.16 (0.12)		0.53 (0.30)	
D3	CP	107 (56)	0	2 (6)	0	4 (6)	45 (12)	226 (31)	0.32 (0.14)	0	0.93 (0.11)	0
	OP	96 (60)			1 (9)		65 (20)	247 (41)	0.28 (0.16)		0.97 (0.23)	
D4	CP	123 (76)	0	10 (14)	8 (10)	22 (20)	116 (25)	565 (47)	0.17 (0.10)	0	0.67 (0.24)	0
	OP	171 (152)			101 (56)		151 (51)	508 (102)	0.22 (0.18)		0.40 (0.28)	
D6	CP	414 (212)	0	11 (22)	31 (22)	9 (17)	104 (28)	940 (115)	0.29 (0.13)	0	0.84 (0.12)	0
	OP	319 (219)			163 (60)		83 (48)	949 (145)	0.22 (0.14)		0.44 (0.20)	
P6	CP	0.56 (0.31)	0.01 (0.06)	0.08 (0.08)	0	0	0.24 (0.08)	1.62 (0.18)	0.24 (0.12)	0.02 (0.11)	0.98 (0.14)	0.13 (0.65)
	OP	0.70 (0.44)			0		0.41 (0.15)	1.30 (0.30)	0.35 (0.19)		1.00 (0.00)	

Correlations between CP and OP breeding value estimates

Despite statistically significant additive genetic variance estimated in both CP and OP cross types in *E. globulus*, genetic correlation between OP and CP ($r_{g(op,cp)}$) breeding values for growth are low and decrease with time (0.30 to –0.10), although no estimate is significantly different from zero (Table 4.7). In contrast, there is a very high correlation for P6 ($r_{g(op,cp)} = 0.95 \pm 0.02$).

In *E. nitens*, the correlation between OP and CP breeding values for growth are high and significantly different from zero, but declined with age (0.83 to 0.50). There is a high correlation for P6 ($r_{g(op,cp)} = 0.82 \pm 0.09$)

Table 4.7 Genetic correlations of OP with CP breeding values ($r_{g\,(op,\,cp)}$) ± standard error (se) for pooled analysis across sites of *E. globulus* and *E. nitens*.

Trait	*E. globulus*		*E. nitens*	
	$r_{g\,(op,\,cp)}$	(se)	$r_{g\,(op,\,cp)}$	(se)
D2	0.30	(0.32)	0.83	(0.15)
D3	0.30	(0.21)	0.83	(0.11)
D4	0.11	(0.25)	0.72	(0.23)
D6	-0.10	(0.23)	0.50	(0.11)
P6	0.95	(0.02)	0.82	(0.09)

Correlations between traits

Table 4.8 Genetic correlation (± standard error) of traits at various ages for CP and OP *E. globulus* (above diagonal) and *E. nitens* (below diagonal) derived from across site analysis.

Trait	Cross type	D2	D3	D4	D6	P6
D2	CP		0.90 (0.02)	0.84 (0.03)	0.80 (0.04)	0.34 (0.09)
	OP		0.95 (0.03)	0.86 (0.07)	0.86 (0.07)	0
D3	CP	0.95 (0.04)		0.97 (0.01)	0.90 (0.02)	0.36 (0.09)
	OP	0.97 (0.03)		0.99 (0.01)	0.91 (0.04)	0
D4	CP	0.89 (0.06)	0.98 (0.02)		0.96 (0.01)	0.40 (0.09)
	OP	1.00 (0)	1.00 (0.01)		0.97 (0.02)	0
D6	CP	0.74 (0.12)	0.87 (0.06)	0.93 (0.03)		0.40 (0.09)
	OP	0.92 (0.10)	0.91 (0.07)	0.99 (0.02)		0
P6	CP	0.59 (0.18)	0.50 (0.19)	0.72 (0.14)	0.77 (0.12)	
	OP	0.68 (0.30)	0.56 (0.33)	0.45 (0.37)	0.87 (0.15)	

The genetic correlation between CP growth traits at different ages is high for *E. globulus* and *E. nitens* (Table 4.8) with correlations highest for traits which are closest in age. OP estimates of genetic correlations were similar in magnitude to CP estimates for both species. In all cases the OP estimate was higher although rarely significantly different from the CP estimate (Table 4.8). The CP genetic correlation between P6 and D6 is lower in *E. globulus* ($r_{g(P6,D6)}$ = 0.40±0.09) than *E. nitens* ($r_{g(P6,D6)}$ = 0.77±0.12) (Table 4.8) with both estimates being significantly different from zero, indicating that faster growing genotypes have generally higher Pilodyn penetration (*i.e.* lower wood density). The genetic correlations between P6 and all growth traits in OP *E. globulus* were never significantly different from zero, whereas CP estimates ranged from 0.34 to 0.40 (Table 4.8) and were all significantly different from zero. In contrast, estimates of correlations between P6 and growth traits in *E.*

nitens were similar in magnitude for both OP and CP. However, OP estimates generally had higher standard errors (Table 4.8).

Discussion

This is the first crossing program in *Eucalyptus* species, where direct comparison of OP and CP genetic parameters using the same parents has been made. It is clear from within site analysis of D6 and pooled analysis across sites for all traits that h^2_{OP} estimates in *E. globulus* are considerably inflated compared to h^2_{CP} estimates. The causes of this bias may be due to unexplained non-additive effects, varying levels of inbreeding within open-pollinated populations and uneven contribution of male parents to the progeny. It should be noted that we have already assumed 30% selfing in the h^2_{OP} calculation to account for differential expression of additive genetic effects (after Squillace 1974) as have a number of other authors (Griffin and Cotterill 1988; Volker *et al.* 1990; Potts and Jordan 1994a; Lopez *et al.* 2001a). However, this adjustment does not account for differential levels of outcrossing or differential expression of inbreeding depression amongst females which may be a major factor causing the inflated variation between OP progenies (Hardner *et al.* 1998).

The results show that *E. globulus* OP material from native stands (GOP) is subject to inbreeding depression of approximately 10% for D6 while the seed orchard open-pollinated material (GSOP) has a lower level at about 2%. These levels of ID for growth are comparable with those obtained in native stand OP *E. regnans* (ID = 6% for diameter at 45 months - Griffin and Cotterill 1988) and *E. globulus* (ID = 13% for diameter at 43 months - Hardner and Potts 1995a). Hardner and Potts (1995a) calculated the average outcrossing rate in *E. globulus* OP was 78% using diameter at 43 months, however most trees used

were ornamental trees growing in a linear planting. They suggest that the frequency of selfing in their study may be higher than found in OP families collected from native forests, but the increase in inbreeding depression appears to be only marginal (13% compared with the present average of 10%). The lower level of ID found in GSOP suggests that the proximity of unrelated trees in the seed orchard is contributing to high levels of outcrossing. In addition, the fact that it proved difficult to produce viable selfed seed through assisted insect pollination or control-pollination suggests that the seed orchard trees used in this experiment may be practically self-incompatible (see Chapter 2).

The single-site h^2_{OP} results for diameter growth in *E. globulus* reported here (D6 average of 0.23 across 5 sites, ranging from 0.11 to 0.32) are comparable with other reports, which range from 0.11 to 0.28 and average 0.20 (Lopez *et al.* 2001a). There appears to be only one other published genetic analysis of growth using control-pollinated progeny in *E. globulus* (Araújo *et al.* 1996). This involved two trials in the south and northwest of Portugal, with full-sib progeny from incomplete crossing among 11 parents. Height h^2 estimates of 0.13 (northwest) and 0.52 (south) were reduced to 0.19 and 0.28 respectively, when parental clonal information was included, however no dominance effects were estimated. In the present trials the CP estimates of h^2 were considerably lower than the corresponding OP estimate from the same site. On average the CP estimates (average 0.12 across 5 sites ranging from 0.07 to 0.18) were nearly half the magnitude of the OP estimates.

The bias of OP genetic parameter estimates in *E. globulus* has serious implications for breeding programs around the world, which have generally been based on genetic parameter estimation based on the use of OP progeny (Volker and Raymond 1989; Volker *et al.* 1990; Woolaston *et al.* 1991a; Borralho *et al.* 1992c; Lopez *et al.* 2001a; Muneri and Raymond 2001). Even for P6,

which has a high level of additive genetic control with very little dominance, the h^2_{OP} estimates were considerably inflated in *E. globulus*. The results presented here demonstrate that estimates of genetic parameters derived from OP seed collected in natural stands of this species cannot be relied upon to produce accurate genetic parameters and are therefore inappropriate for advanced breeding.

Inflated estimates of heritabilities derived from OP mean that genetic gains for growth as a result of breeding operations will be over-estimated. Genetic gains for growth using OP material have previously been estimated by Volker *et al.* (1990) and Woolaston *et al.* (1991a). Parental selection based on OP progeny performance clearly is not reflecting the true breeding value of the female parent and most likely reflects variation in the pollen environment (Borralho and Potts 1996), self-incompatibility or level of genetic load (Hardner and Potts 1997) of the parent. The OP performance for diameter at all ages in *E. globulus* is clearly a different trait than for CP, as indicated by their low genetic correlation. The low genetic correlation obtained between OP and CP, contrasts with the significant correlation obtained between close and wide outcrosses for the same *E. globulus* parents (range 0.60 to 0.97) (Chapter 5: Table 5.8).

The discrepancies in OP estimates are most likely due to variation in inbreeding depression amongst families (Griffin and Cotterill 1988; Hardner and Potts 1995a; Hardner *et al.* 1998). Variation in outcrossing rate is believed to affect not only the expression of additive genetic effects but also the level of inbreeding depression. Hardner *et al.* (1996) have shown considerable variation in outcrossing rates amongst native stand females of *E. globulus*, with low outcrossing rates tending to be associated with poor performance of the OP progeny. A similar trend was also observed in *E. grandis* (Burgess *et al.* 1996). These results suggest that the precision of predicted breeding values

using OP progenies may be improved by adjusting family performance by individual outcrossing rate. The present result shows that reliable genetic parameters for growth can only be obtained through full outcrossing of unrelated individuals. This can be achieved through control pollination, which has become practically feasible on a relatively large scale using single visit pollination procedures (Harbard *et al.* 1999; Williams *et al.* 1999). Alternatively, better correlations may be possible in multi-provenance or family seed orchards where the pollination environment is more uniform and there is a substantial overlap in flowering time between unrelated individuals.

In *Eucalyptus,* Pilodyn penetration has consistently shown high genetic or phenotypic correlation with wood density (Dean *et al.* 1990; Greaves *et al.* 1996; Greaves *et al.* 1997b; Raymond and MacDonald 1998; Muneri and Raymond 2001; Raymond and Muneri 2001), but its heritability seems to vary with the species and site conditions (MacDonald *et al.* 1997; Muneri and Raymond 2001; Raymond and Muneri 2001). The only other estimates of heritability for Pilodyn in *E. nitens* was $h^2_{OP} = 0.50$ across two open pollinated trials, by Greaves *et al.* (1996) and $h^2_{CP} = 0.42$ for 9 year old control-pollinated material studied by Hardner and Tibbits (1998). The Pilodyn heritabilities presented here for *E. nitens* ($h^2_{OP} = 0.35$) are in line with estimates reported by Tibbits and Hodge (1998) for a direct measure of basic density using cores in open-pollinated progeny trials at age 6 years across six sites. In this case the average h^2_{OP} was 0.43. The present result is consistent with another finding of no dominance variance for Pilodyn (Hardner and Tibbits 1998). Pilodyn heritability estimates in *E. globulus* OP material have been reported and range between 0.13 and 0.57 (Dean *et al.* 1990; MacDonald *et al.* 1997; Muneri and Raymond 2001; Raymond and Muneri 2001). The overall average for *E. globulus* in this study was at the high end of this range ($h^2_{OP} = 0.61$) and clearly higher than growth estimates, which is consistent with wood density being of

higher heritability than growth (MacDonald *et al.* 1997; Muneri and Raymond 2001).

In contrast to the results obtained for growth, the OP and CP breeding values for P6 in *E. globulus,* which have a high heritability, appear to be highly correlated, although the OP heritability is significantly over-estimated in comparison to CP. Significant correlations in *E. globulus* between OP and CP performance have also been reported from this trial for *Mycosphaerella* leaf disease (Dungey *et al.* 1997) and timing of vegetative phase change (Jordan *et al.* 1999). In each case, there is a high genetic correlation between OP and CP performance indicating that the same genes are influencing performance. Such traits are unlikely to be influenced by inbreeding depression and for such traits, and other traits of high heritability, selection based on OP is likely to be reasonably accurate and a practical means of breeding.

The discrepancy in growth–Pilodyn genetic correlations between OP and CP *E. globulus* is noteworthy and potentially has important implications for tree breeding strategies. It has been assumed that the key selection traits, growth and wood density (measured indirectly by the Pilodyn), are not genetically correlated and can be improved independently (Lopez *et al.* 2001b). However, estimates of these parameters based on OP material suggest a slight positive correlation between growth and Pilodyn ranging from -0.04 to 0.85 (MacDonald *et al.* 1997; 2001a; Lopez *et al.* 2001b; Muneri and Raymond 2001), however the majority of these reported estimates are not significantly different from zero. A positive correlation is adverse for the breeding objective which seeks to increase growth and basic density simultaneously (Greaves *et al.* 1997a). The present results ($r_{g(D6\text{-}P6)}$ = 0 and 0.40 for OP and CP respectively: Table 4.8) suggest the true strength of the adverse genetic correlation between these traits may have been underestimated in OP, if it is accepted that the CP estimate is more reliable.

Published reports of quantitative genetic parameters in *E. nitens* show h^2_{OP} for diameter between 0.18 and 0.39 (Whiteman *et al.* 1992; Kube *et al.* 1996; Kube and Raymond 2002), and 0.47 and 0.42 for summer and winter Pilodyn penetration respectively (Kube and Raymond 2002) across a range of trials in southeastern Australia and New Zealand. Two New Zealand provenance/progeny trials using OP progeny from native forests (with coefficient of relationship set at 0.5) and plantations or seed orchards (coefficient of relationship set at 0.25), assessed at age 5 and 6 years, gave a h^2_{OP} of 0.11 for diameter and 0.41 for Pilodyn penetration with a genetic correlation of -0.14 between the traits (Gea *et al.* 1997). Kube and Raymond (2002) found genetic correlations of diameter, with summer and winter Pilodyn traits of 0.52 and 0.63 respectively, which are similar in magnitude to the genetic correlation of diameter at various ages with Pilodyn in this study (0.45 to 0.87). In the present study *E. nitens* h^2_{OP} for D6, in across-site analysis, was 0.22 and P6 was 0.35 (Table 4.6), which is comparable with the above studies. The present study shows that *E. nitens* h^2 estimates are similar in OP and CP for within site analysis of D6 at WR and in the across site analysis of all traits. There is also a high correlation of breeding values across sites.

It is clear from the above that the results for *E. nitens* contrast with those obtained for *E. globulus*. This may be due to differences in the genetic architecture of the two species (*e.g.* this population of *E. nitens* is not subject to as severe inbreeding depression) or may be due to specific attributes of the genetic material studied, including differences in pollination environment. *E. nitens* has been reported as exhibiting comparable inbreeding depression for growth as *E. globulus* and other eucalypt species (Hardner and Tibbits 1998). The *E. nitens* OP material in the present study was obtained from trees located in seed orchards and plantations. It is therefore most likely that the *E. nitens* OP material used in this study resulted from a higher level of outcrossing than

would be expected from native stand material. In addition, the difficulty in producing viable selfed seed in these specific *E. nitens* parents suggests they are practically self-incompatible. Despite little or no evidence of inbreeding depression in the least squares means obtained for any trait with *E. nitens* there was still some disparity between OP and CP genetic parameter estimates, however the sample size is quite small being progeny from only 10 parents, so results should be treated with some caution.

Dominance effects (d^2) were generally quite small in both species, but in *E. globulus*, at least, were comparable to additive genetic effects expressed as narrow-sense heritability (h^2) in single site analyses of growth. Additive and dominance effects on growth were also generally comparable in *E. regnans* (Griffin and Cotterill 1988) and *E. nitens* (Hardner and Tibbits 1998) when CP progeny were grown at a single site. However, in the case of *E. globulus*, the expression of these dominance effects was site specific and across site estimates were not significantly different from zero and markedly less than additive genetic effects. The level of dominance variation for Pilodyn was generally much smaller than additive genetic variation in the present study for both species. Hardner and Tibbits (1998) also reported dominance effects on wood density of *E. nitens* were low compared with additive genetic effects.

The low across site levels of dominance for growth are somewhat at odds with the observed levels of inbreeding depression evident in OP progeny when compared with CP progeny. A change in population mean following inbreeding depression should depend to a fairly large extent on the level of dominance variance (Mayo 1987; Falconer and MacKay 1996). However, inbreeding depression may also be due to the exposure of deleterious mutations which are carried as the heterozygote where the effect is masked, such as reported in *E. gunnii* (Potts 1990). If these mutations are rare in the population, then crossing among unrelated individuals (particularly those

from different populations) may not unite deleterious recessive alleles and hence such dominance will not be apparent under outcrossing (Hardner and Tibbits 1998).

Genotype by environment interactions (gxe) appear to be under-estimated in OP compared with CP for growth traits in *E. globulus* (Table 4.5), whereas in *E. nitens* gxe appears to be over-estimated in OP (Table 4.6). In the case of *E. globulus* this may well be due to a family stability induced by differences in inbreeding, for which the deleterious effects are consistent across sites. The gxe exhibited by both species for P6 is comparable between OP and CP and quite low. This indicates that gxe estimates derived from OP growth should be treated with some caution. In *E. nitens*, Kube *et al.* (1996) found high genetic correlation in performance of OP families across a range of sites in Australia and New Zealand, suggesting that diameter growth could be considered as a single trait and that the species has negligible gxe across regions. A similar finding was made across three 12-year-old *E. nitens* OP progeny tests for diameter and Pilodyn traits in Tasmania (Kube *et al.* 2000). Breeders faced with the choice of a single breeding population or multiple breeding populations for a range of sites require reliable and unbiased estimates of gxe. The consequences of making the wrong decision can have serious implications in terms of genetic gain, time and cost of the breeding program.

Conclusion

The present study suggests that estimates of the amount of additive genetic variation for growth traits in native stand OP *E. globulus* populations available for selection may have been over-estimated. Furthermore, genetic variation amongst native stand OP families appears to be poorly correlated with

variation in breeding values amongst the female parents. Similar results have been reported in *E. regnans* (Griffin and Cotterill 1988) and *E. nitens* (Hardner and Tibbits 1998). Genetic evaluation for growth based on native stand OP progenies therefore appears to be unreliable. In contrast, OP progenies appear to provide a good prediction of parental breeding values for traits such as Pilodyn (an indirect measure of wood density). Whether such inconsistencies arise in seed orchard OP progenies is unclear at present. The results reported here suggest that in such circumstances, inbreeding may be less and at least in the case of *E. nitens,* the OP progeny derived from seed orchards containing unrelated ortets and plantations derived from multiple unrelated parents may better reflect the variation in parental breeding values. In addition, the genetic correlation between growth and Pilodyn in OP is completely different from the result obtained with CP material in *E. globulus,* while in *E. nitens* the correlations are comparable between cross types. In the case of *E. globulus,* there is a strong argument for the rapid adoption of genetic evaluation through control-pollination programs. These results support the argument that selection from OP base populations may lead to sub-optimal gains as poor growth performance under OP may mask a high breeding value of a parent or individual (Potts *et al.* 1995b). Finally, the unreliability of additive genetic parameters derived from native stand OP *E. globulus* for growth also appears to distort the genetic correlation between growth and Pilodyn. These two traits are very important in breeding objectives for kraft pulp production (Greaves *et al.* 1997a).

Despite relatively low levels of dominance variance within both species it has been shown that inbreeding effects may still bias OP genetic parameter estimates, particularly in *E. globulus.* The dominance effects in *E. globulus* are comparable to the additive genetic effects and are highly site specific. The

latter finding adds a further complication to the exploitation of SCA effects and choice of parents for use on particular sites.

The accuracy of selection and prediction of genetic gains in a species is determined by the confidence that can be placed in genetic parameter estimates and the estimation of breeding values. This study, combined with other work on the species, has shown that OP progenies from native stands in *E. globulus* do not provide accurate genetic parameter or breeding value estimates for growth. Further weight is added to the argument that results are confounded by differential levels of inbreeding at the tree and population levels from material which has been sourced from native stands. The available evidence suggests that this problem may not be as severe in the *E. nitens* population tested, which was derived from seed orchards and plantations of known genetic origin, however larger crossing programs are required in this species to test this hypothesis.

Chapter 5: A comparison of genetic parameters of intra- and inter-specific hybrids of *Eucalyptus globulus* and *E. nitens*

Introduction

Eucalypts are the most widely planted hardwood genus in the world (Eldridge *et al.* 1993). Their rapid expansion into new environments, combined with their ability for inter-specific hybridisation (Griffin *et al.* 1988; Potts and Wiltshire 1997), has led to the development of many commercial hybrids (Martin 1989; Sedgley and Griffin 1989; Potts and Dungey 2001). In particular, hybrids of the sub-tropical eucalypts including various combinations of *E. grandis, E. urophylla, E. tereticornis* and *E. camaldulensis* have been used in forestry for many years in India, Brazil, Congo, Morocco and South Africa through use of seed or cuttings (Martin 1989; Eldridge *et al.* 1993). Many early selections were often from sporadic hybrids, occurring in seed collections derived from native stands or exotic species trials (see Eldridge *et al.* 1993). There is now increasing interest in specifically breeding hybrids (Martin 1989; Vigneron 1991; Nikles 1992; Nikles and Griffin 1992; Vigneron 1995; Shelbourne 2000; Vigneron and Bouvet 2000), particularly F_1's, although the rationale and the genetics of hybrid populations are poorly understood.

Hybrid superiority is usually attributed to heterosis or complementarity of traits (Nicholas 1987; Falconer and MacKay 1996). Heterosis occurs as a result of non-additive (mostly dominance) gene actions affecting the trait of interest. Complementarity, on the other hand, is obtained through additive effects and

results from synergy amongst independent traits in specific environments (*i.e.* non-linear effects such as genotype by environment interaction) where both parent species are less well adapted than their hybrid (Nicholas 1987; Martin 1989; Sedgley and Griffin 1989; Nikles and Griffin 1992). The importance of heterosis is a key issue in the exploitation of hybrids in forestry (Nikles and Griffin 1992). If hybrid superiority is shown to be the result of true genetic heterosis, dependent upon dominance or over-dominance effects (Ledig 1986; Klekowski 1988), this would open the possibility of its exploitation by crossing highly differentiated populations (Nicholas 1987; Nikles 1992). Unfortunately, this approach may be countered by the potential for outbreeding depression arising from incompatibilities between markedly divergent genomes (Templeton *et al.* 1986; Orr 1995), the potential increasing with increasing taxonomic distance between parents (Griffin *et al.* 1988; Ellis 1991; Baril *et al.* 1997a; 1997b; Potts and Dungey 2001). Not surprisingly, there have been conflicting results when relating genetic distances with the presence of heterosis. However, in forest trees it has been argued that hybrid superiority is unlikely to result from heterosis, but is more likely to be the result of beneficial combination of independent complementary traits (Ledig 1986; Martin 1989; Nikles 1992; Nikles and Griffin 1992; Vigneron and Bouvet 2000).

There are many reports of superiority of inter-specific F_1 hybrids of *Eucalyptus* mostly from India (Venkatesh and Sharma 1976; 1977b; Venkatesh and Sharma 1977a; Venkatesh and Sharma 1979; Venkatesh and Thapliyal 1993; Paramathma *et al.* 1997), Brazil (Campinhos and Ikemori 1977; 1989; Kageyama and Kikuti 1989; Blake and Bevilacqua 1995; Wright 1997), Congo (Wright 1997; Vigneron and Bouvet 2000) and South Africa (Darrow 1995; Wright 1997). However, intra-specific controls are often absent, or of poor accuracy (*i.e.* open-pollinated or unrelated to the F_1's). This lack of adequate controls makes it difficult to assess whether differences between hybrid and

pure species is simply a result of removing inbreeding effects. The question remains whether comparable genetic gains could have been achieved simply through removing inbreeding effects through wide intra-specific outcrossing and selection within species (Eldridge *et al.* 1993).

Forestry programs using eucalypt hybrids generally rely on phenotypic selection of individuals for propagation. This is followed by a period of performance evaluation of propagules before a particular clone is produced on a commercial scale. Knowledge of genetic parameters of hybrid populations has the potential to reduce the time taken for these steps and to return more information to the tree breeder to improve accuracy of selection for propagation and breeding.

There are reports of genetic parameters for inter-specific hybrid populations estimated for breeding and deployment purposes (Bouvet and Bailleres 1995; Bouvet and Vigneron 1995; 1996a; Bouvet and Vigneron 1996c; 1996b; Dungey *et al.* 2000a; Dungey and Nikles 2000; Gwaze *et al.* 2000). However, the general lack of pure species controls means that there is little information on whether hybrid populations behave similarly to pure species populations and conform to current quantitative genetic models. Such fundamental information is necessary if F_1 hybrid production is to proceed beyond haphazard crossing so that rigorous strategies can be developed for breeding improved hybrids. A key issue is whether reciprocal or simple recurrent selection schemes are most appropriate for breeding superior F_1 hybrids (Nikles 1992; Nikles and Griffin 1992; Baradat *et al.* 1994; Bouvet and Vigneron 1996b; Dungey *et al.* 2000c; Kerr *et al.* 2000; Shelbourne 2000). Reciprocal recurrent selection (RRS) involves crossing amongst pure species parents following their backward selection after testing in hybrid combination. Alternatively, simpler schemes of recurrent selection (RS) based on performance within pure species breeding populations and crossing amongst elite selections are attractive as they offer

considerable time and cost saving and flow directly from pure species improvement programs (Dungey *et al.* 2000c; Kerr *et al.* 2000).

Differentiation of these breeding strategies depends on whether the best pure species selections also produce the best hybrid combinations or whether F_1 hybrid performance is unrelated to the General Combining Ability (GCA) of the parent in pure species combination. Nikles and Newton (1991) raise the concept of General Hybridising Ability (GHA) as a measure of the 'additive' performance of a trait in hybrid combination. Which strategy is most suitable will, to a large extent, depend on the correlation between GCA and GHA estimates (tropical pine hybrids -Nikles and Newton 1991; Powell and Nikles 1996) and, more precisely, the genetic correlation between pure species and hybrid performance (Dieters and Dungey 2000; Newman and Reverter 2000) and their relative variability in the pure species and hybrid populations respectively. Similarly, predictability of which parental combination will produce the best F_1 hybrids for deployment, whether by seed (full-sib families) or clonal propagation, will dependent upon the magnitude of specific combining effects in hybrid crosses, termed Specific Hybridising Ability (SHA;) (Nikles and Newton 1991).

Eucalyptus globulus is a forest tree native to south-eastern Australia (Jordan *et al.* 1993; Dutkowski and Potts 1999). It is the premier eucalypt for the Kraft pulping process, because of its high pulp yield and wood density, which are both strongly, correlated to wood costs in the chemical pulping processes (Greaves and Borralho 1996; Greaves *et al.* 1997a). It is widely planted in temperate regions of the world (*e.g.* Australia, California, Chile, China, Italy, Portugal and Spain) for pulpwood production (Borralho 1992; Eldridge *et al.* 1993; Tibbits *et al.* 1997a), but plantations are confined to relatively frost free areas (Tibbits *et al.* 1991a; 1997a). The more frost tolerant species, *E. nitens*, is often used as a replacement for *E. globulus* in plantations on colder sites

(Tibbits *et al.* 1989; 1991a; 1997a; Tibbits and Hodge 2001). It has a wide but discontinuous geographic distribution in eastern Australia (Pederick 1979; Tibbits and Reid 1987a). Material sourced from central Victorian provenances (Dutkowski *et al.* 2001) is of interest for crossing with *E. globulus* to produce frost resistant, fast growing hybrid trees with superior pulp properties suitable for planting in cool temperate environments such as those found in Tasmania, and Chile and higher altitude areas in warmer climates. *E. nitens* shows a featured grain in timber or veneer because of distinct differences between early- and late wood (Nicholls and Pederick 1979), whereas *E. globulus* tends to have a more uniform colour and texture (Hillis and Brown 1984). The pulping features of *E. nitens* for the Kraft process are promising (Dean 1995), but for different paper sectors than *E. globulus* (Cotterill and Brolin 1997). Nevertheless, the species is yet to be widely accepted as top pulpwood commodity (Rojas Vergara *et al.* 2001).

While there is certainly the opportunity to improve density, growth and frost resistance by exploiting the genetic variation within both *E. globulus* (Dutkowski and Potts 1999) and *E. nitens* (Tibbits and Hodge 1998), there is considerable interest in developing hybrids between these two species (Espejo *et al.* 1995; Volker 1995; Tibbits *et al.* 1997a; Rojas Vergara *et al.* 2001). Indeed, when a breeding or deployment objective of improved growth and increased density is considered, coupled with the opportunity to increase frost resistance (but see Chapter 3), the hybrid of *E. nitens* and *E. globulus* has been proposed as an ideal combination. For pulp and paper production a higher density in combination with higher growth is generally desirable (Greaves and Borralho 1996).

E. nitens and *E. globulus* are difficult to clonally propagate economically, although some success has been achieved with the latter species (Wilson 1993; England and Borralho 1995; Wilson 1996; 1998; 1999). The F_1 hybrid can only

be produced using the smaller flowered *E. nitens* as the female (Gore *et al.* 1990) and vegetative propagation also appears to be difficult (Rasmussen *et al.* 1995). *E. globulus* and *E. nitens* are relatively closely related (Subgenus *Symphyomyrtus*, Section *Maidenaria*, Series *Viminales*; (Pryor and Johnson 1971) or Series *Globulares* (Brooker 2000)), yet the F_1 seed produce high levels of abnormal, dwarf-like seedlings (Potts *et al.* 1992; Espejo *et al.* 1995; Potts *et al.* 2000). Despite these problems the many possible complementary features of *E. nitens* and *E. globulus* have maintained world-wide interest in development of their F_1 hybrid to extend the range of "*globulus*-like" plantations.

This chapter compares the performance and genetic parameters of inter-specific F_1 hybrids between *Eucalyptus nitens* and *E. globulus* ssp. *globulus* and intra-specific crosses grown on the same site. The crossing design is unique in studies of eucalypt hybrids to date, due to (i) the relatively large number of parents involved, (ii) the maintenance of common parentage in pure species and hybrid crosses and (iii) the generation of both inter-provenance and inter-specific hybrids. This design allowed the comparison of inter-specific F_1 hybrid performance without appreciable inbreeding in the pure species controls, estimates of additive and non-additive genetic variance for intra-provenance, inter-provenance and inter-specific crosses and a direct comparison of parental performance in hybrid and pure species combination. The present study extends the studies of the genetics of frost (Chapter 3) and disease resistance (Dungey *et al.* 1997) in an inter-specific F_1 hybrid population of *E. nitens x globulus*. This study reports on the genetics of key breeding traits, growth at ages 2, 3, 4, 6 and 10 years and wood density, at 6 years of age at a single site.

Materials and Methods

Mating designs

A controlled crossing program was undertaken by CSIRO Division of Forestry and North Forest Products in 1987 to 1989 to generate intra- and inter-specific crosses of *E. globulus* and *E. nitens* with common parentage (see Chapter 2). A virtually complete factorial crossing design was used for *E. globulus* (Table 5.1). Twenty-six unselected male parents from native stands at Taranna (16 parents) and King Island (10 parents) were mated with seven selected female parents derived from the same provenances (4 from Taranna and 3 from King Island).

The control-pollinated *E. nitens x globulus* F_1 hybrids were derived from an incomplete factorial mating design (Table 5.1) using 14 of the 26 *E. globulus* male parents and 7 of 11 *E. nitens* parents.

The control-pollinated *E. nitens* plants were derived from an almost complete half diallel mating (Table 5.1) of 10 first generation parents from provenance, Toorongo.

Cross types are denoted in the text as intra–provenance crosses in *E. globulus*: TT (Taranna x Taranna), KK (King Island x King Island); inter-provenance crosses (or intra-specific hybrids) in *E. globulus*: TK (Taranna x King Island), KT (King Island x Taranna); inter-specific F_1 hybrids: NT (*E. nitens* x Taranna *E. globulus*), NK (*E. nitens* x King Island *E. globulus*) and NN (intra-specific crosses in *E. nitens*).

Field Trial Location and Design

In July 1990, a trial was established by CSIRO Division of Forestry and North Forest Products on an ex-pasture site at West Ridgley, Tasmania (latitude 41°09′ longitude 145°46′; altitude 185m). Soil and climatic details are given in Dungey *et al.* (1997). The trial was established using a resolvable incomplete block design, otherwise known as an alpha design (Paterson and Williams 1976). There were 20 plots per incomplete block comprising 14 *E. globulus*, 3 hybrid and 3 *E. nitens* plots. Within each incomplete block, the *E. globulus*, *E. nitens x globulus* and *E. nitens* were planted in separate sub-blocks to minimise competitive effects between cross types. There were 15 incomplete blocks per replicate and 4 replicates in the trial. Trees from each family were planted in five tree line-plots and spaced at 3 x 3m. The *E. globulus* and *E. nitens* material included controlled pollinated (CP) and open-pollinated (OP) material as well as *E. globulus* inter-provenance crosses with a female parent from south Flinders Island (Table 2.1).

Table 5.1 The number of individuals from each family planted at the West Ridgley Trial for *E. globulus* intra- and interprovenance crosses, *E. nitens x globulus* F_1 hybrids and *E. nitens*. (T=Taranna, K=King Island, TO=Toorongo)

Female parents		*E. globulus* male parents																								
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	K17	K18	K19	K20	K21	K22	K23	K24	K25	K26
E. globulus	K-a	25	20	24	18	20	23	25	16	20	22	32	20	23		24	20	20	22	20	19	20	20	25	27	20	20
	K-b	35	20	45	28		18	15	20	20	19	20	17	19	20	28	33	14	21		20	20	17	25	13	20	20
	K-c	20	13				20	12	13	20	12	24	11	22	20	20	14	19	20	20	20		20	21	20	23	20
	T-e	18	19	10			20	20	13	20		20	20		15	20	20		18		16						
	T-f	23	20	20	20	20	20	20	20	23	20	20	20	22	20	14	20	31							19		15
	T-g	18	20	20	15	20	20	20	20	20	20	25	19	20	20	20	20	25	20	20	23	20		20	29	28	
	T-h	29	20	17			20		20		20	20	20	14	22		20		20	20		28				20	
E. nitens	TO-i																										
	TO-j																										
	TO-k				7		17				19	6	7				9				12		8				11
	TO-l				20		20					13									17		13				22
	TO-m	16			21		17				15	20				8					12					17	11
	TO-n				21		23					11	14	22		18					7						16
	TO-o				20		5				15	18	9			23					10						20
	TO-p	20				13																					
	TO-s							20																			

	E. nitens male parents							
	TO-k	TO-l	TO-m	TO-n	TO-o	TO-p	TO-q	TO-r
E. globulus								
E. nitens	20		17	17		20		20
		24	34	15		17	20	17
		14	20		18		18	17
			20	23	28	21	24	25
				20		20	20	20
					25		19	11
			25			20	19	13
							20	20

Measurement

Trials were measured at 2, 3, 4, 6 and 10 years from planting in the winter of each year. The diameter at breast height (1.3m above ground level) over bark (DBHOB - mm) was measured as well as an indirect measure of density obtained using a Pilodyn. The Pilodyn is an instrument which shoots a pin into the tree, with increased penetration of the pin correlating with lower wood density (Greaves *et al.* 1996; Raymond *et al.* 1998). In 1996 at age 6 years, the first three healthy trees in each plot were assessed for Pilodyn penetration. The Pilodyn assessment followed the method described in Raymond and MacDonald (1998), where two readings were taken from a single window made by removing the bark and the readings averaged. Previous analyses have shown a very high correlation between DBHOB and conic volume at these ages (r^2 greater than 0.95; P. Volker unpubl. data) thus, for simplicity and comparability with other studies (MacDonald *et al.* 1997) and breeding programs (Jarvis *et al.* 1995), DBHOB is the trait used as an indication of growth. These DBHOB traits at each year are described as D2, D3, D4, D6, D10 and the Pilodyn trait is P6.

Analysis

Analysis of the data was carried out all surviving trees at each measurement, even those trees which could be described as deformed, non-vigorous individuals, dead trees were treated as missing values in the analysis. All OP and CP material from Flinders Island was excluded from this analysis.

The data was analysed using univariate and multivariate individual tree mixed models with ASREML (Gilmour *et al.* 1999). ASREML fits the general mixed model

$$y = \mathbf{X}\beta + \mathbf{Z}\mu + \varepsilon \qquad \text{(Equation 5.1)}$$

where y is a vector of observations, **X** is the design matrix for fixed effects β, **Z** is the design matrix for random effects $\mu \sim (0, \sigma^2\mathbf{G})$, and ε $(0, \sigma^2\mathbf{R})$ are residuals. ASREML has the ability to utilise multi-trait information in a genetic analysis with a relationship matrix (**A**) in the definition of **G**. ASREML uses the average information algorithm and sparse matrix technology to calculate restricted maximum likelihood variance and covariance components (Gilmour *et al.* 1999). A more complete description of ASREML analysis procedure is given in Chapter 3. A matrix of relationships (a pedigree) for each tree was used to identify the additive genetic effects (Gilmour *et al.* 1999). The model used to estimate cross type effects and levels (and significance) of mid-parent heterosis included replicate and cross type (TT, KK, TK and KT, NT, NK and NN; see Table 5.2) as fixed effects. The inter-provenance crosses TK and KT were treated as a single cross type. The random effects were the additive genetic effects of individuals and their parents (female and male), the female by male interaction effects within cross types, the incomplete block within replicate and the plot effects. The treatment of cross type effect as a fixed effect is equivalent to their treatment as genetic groups as far as parameter estimation is concerned.

Pooled genetic parameters for *E. globulus* overall were estimated by fitting the same model, but excluding crosses involving *E. nitens*. Parameters within only the inter-specific F_1 hybrid crosses were estimated with the same model with two levels of cross type (NT and NK). The cross type effect was excluded from the model for estimating genetic parameters separately for *E. nitens* and the three *E. globulus* cross types (TT, KK, pooled TK and KT). The female by male interaction estimated either SCA in pure species crosses or SHA in F_1 hybrids. Narrow sense heritabilities (h^2) were calculated as:

$$h^2 = \frac{\sigma^2_{add}}{\sigma^2_{add} + \sigma^2_{sca} + \sigma^2_{plot} + \sigma^2_{res}} \qquad \text{(Equation 5.2)}$$

where σ^2_{add}, σ^2_{SCA}, σ^2_{plot} and σ^2_{res} are the additive, SCA (or SHA in the case of the F_1 hybrids), plot and residual variances respectively. An estimate of relative significance of dominance (assumes no higher order genetic interactions such as epistasis) was calculated as

$$d^2 = \frac{4\,\sigma^2_{sca}}{\sigma^2_{add} + \sigma^2_{sca} + \sigma^2_{plot} + \sigma^2_{res}} \qquad \text{(Equation 5.3)}$$

The denominator in equations 5.2 and 5.3 is the phenotypic variance (σ^2_P).

Coefficients of variation such as coefficient of additive variation (CV_A) and coefficient of phenotypic variation (CV_P) were calculated using the appropriate variance component and least square means as:

$$CV_x = \left(\sqrt{\sigma^2_x}/\mu_x\right) \times 100 \qquad \text{(Equation 5.4)}$$

where CV_x is coefficient of variation of parameter x expressed as a percentage, σ^2_x is the variance component and μ_x is the least squares mean.

F_1 hybrid heterosis was calculated as the difference between hybrid mean performance and the mean of the parental lines, *i.e.* mid-parent heterosis (Mayo 1987).

$$H_{(1,2)} = F_1 - \left(\frac{(P_1 + P_2)}{2}\right) \qquad \text{(Equation 5.5)}$$

where $H_{(1,2)}$ is the estimate of heterosis for the hybrid between species or provenances 1 and 2, F_1 is the mean for the trait in the F_1 hybrid (within

species or between species), P_1 and P_2 are the trait means for each trait in the parental species or provenances. This value was also expressed as a percentage of the mid-parent value. In this case heterosis was calculated for intra-specific F_1 hybrids in *E. globulus* (*i.e.* crosses between Taranna and King Island provenance with KT and TK treated as one cross type), and for inter-specific F_1 hybrids (*E. nitens x globulus*) where the provenance of the *E. globulus* parents was differentiated into Taranna (NT) and King Island (NK). The tests of significance of the deviation of the F_1 from the mid-parent value were undertaken using a family model (replicate, incomplete block within replicate, male, female, male by female interaction as random terms) with the contrast option in PROC MIXED of SAS Version 8.

Genetic correlations between traits within cross types were estimated using bivariate analyses with ASREML. The genetic correlations and their standard errors were estimated directly in the model using the CORR function in ASREML. This uses a Taylor series approximation to derive standard errors (Gilmour *et al.* 1999).

Genetic correlations between the performance of parents in pure breed and hybrid combination were determined using the program ASREML (Gilmour *et al.* 1999) as follows. In the analyses a trait measured in a different cross type was treated as different trait (i.e. D2 for TT was treated as a separate trait to D2 for TK etc.). This approach was adopted for both intra- (TK and KT) and inter-specific hybrids (NT and NK) and was done for all the growth (D2, D3, D4, D6, D10) and Pilodyn (P6) measurements. The genetic correlation between pure species and hybrid performance is a direct estimate of the correlation of GCA and GHA described earlier. This genetic correlation is estimated with pedigree linkages using either the common male parents for the inter-specific hybrid or common male and female parents for the intra-

specific hybrids. The analyses were undertaken in two stages. First, within *E. globulus*, using the inter-provenance hybrids (TK and KT treated as one cross type) and intra-provenance crosses (TT and KK) as separate cross types and therefore, separate traits in bi-variate individual tree mixed model analysis, excluding plot and SCA effects. Second, within *E. globulus* cross-types were treated as fixed effects to pool the *E. globulus* data into a single trait, with the multi-variate individual tree model analysis including *E. nitens* and *E. nitens x globulus* (NT and NK treated as separate cross-types) as separate traits and excluding plot and SCA effects from the model. In this case, genetic correlation refers to the correlation of genetic effects within cross types. Parameter estimates that differed by more than 2 standard errors from zero were treated as significant (Gilmour *et al.* 1999) as detailed previously.

Results

Performance and heterosis at the cross type level

The mean performance of cross types for all traits is presented in Table 5.2. Cross type survival is shown in Figure 5.1 and Table 5.3. Heterosis of the *E. globulus* inter-provenance (intra-specific) and *E. nitens* x *globulus* inter-specific F_1 hybrids, expressed as deviation from the mid-parent value is shown in Table 5.4. The individual frequency distribution of diameter for the three CP cross types (GCP, F_1 hybrid and NCP) for ages 2, 6 and 10 years is shown in Figure 5.2.

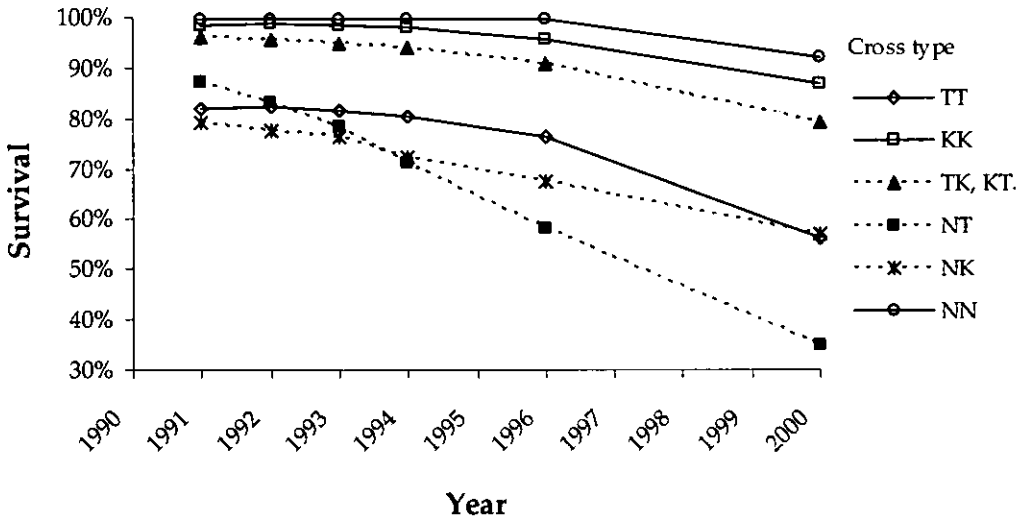


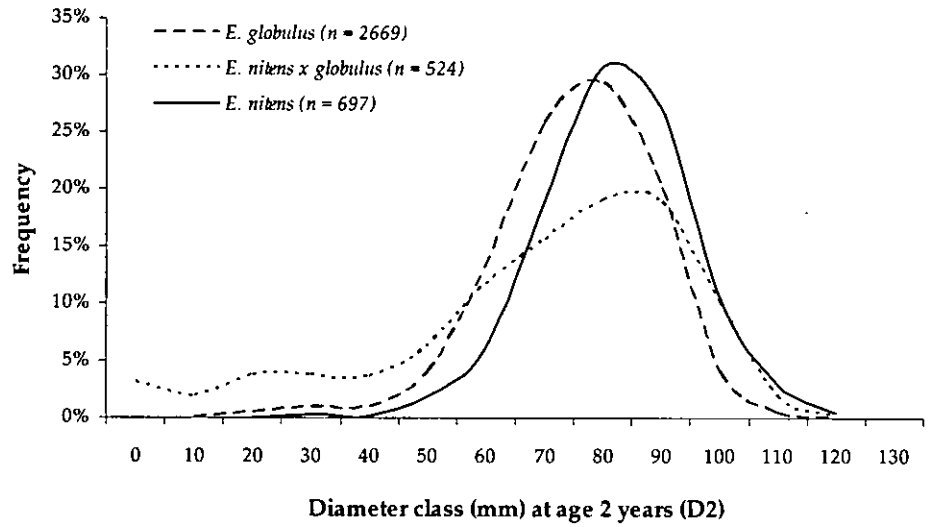
Figure 5.1 Survival of cross types in West Ridgley Trial expressed as percentage of number of trees planted in 1990. Cross types are *E. globulus* intra-provenance (TT, KK), *E. globulus* inter-provenance (TK, KT) , *E. nitens x globulus* (NT, NK) and *E. nitens* (NN).

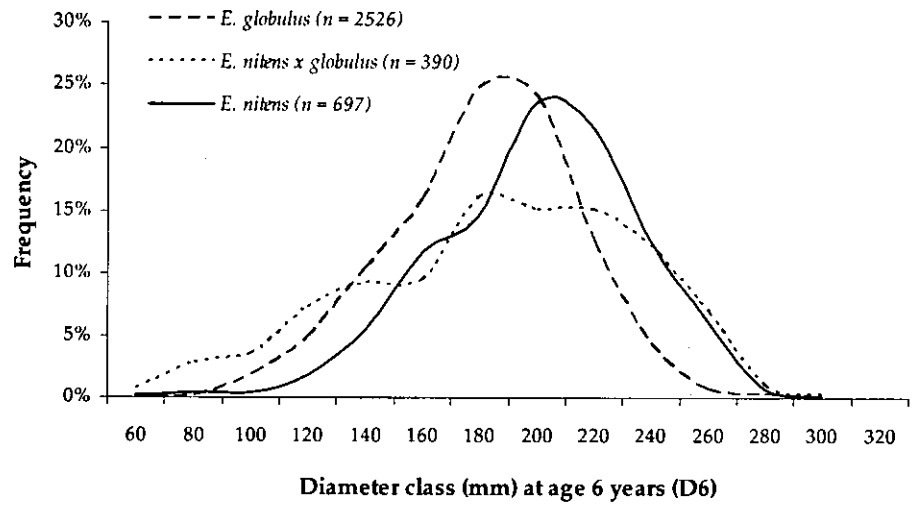
The growth (Table 5.2) and survival (Fig. 5.1 and Table 5.3) of *E. nitens* was better than *E. globulus* at this site, with its superiority increasing with age (Fig. 5.1, Table 5.2). Within *E. globulus* the King Island intra-provenance crosses (KK) grew better (Table 5.1) and had better survival (Fig. 5.1) than Taranna intra-provenance (TT) at all ages. Survival of the inter-provenance hybrids (TK, KT) was effectively intermediate between the intra-provenance crosses (KK and TT) (Fig. 5.1). Survival of the interspecific hybrids (NT, NK) was markedly less than any of the *E. globulus* or *E. nitens* pure species crosses (TT, KK, KT, TK and NN) by age 6 years (Fig. 5.1, Table 5.3). Cross types involving Taranna parents (TT, NT, TK and KT) had lower survival (Table 5.3; Fig. 5.1) and reduced vigour (Table 5.2) at all ages compared with KK and NN, and the survival of TT crosses by age 10 was as low as the NK inter-specific hybrids (Fig. 5.1).

At the family level, survival was highly variable at age 6 years, particularly in the F_1 hybrids where families ranged from 0 to 100% (Table 5.3). This was mainly due to specific males, which consistently produced inviable families in hybrid combination. For example, the Taranna male T11 produced five hybrid families, which had a maximum survival of 33% with two failing completely. Similarly the five families produced by Taranna male T6 had a maximum survival of 35%. In both these cases the overall survival for the males was 10 and 24% respectively. This contrasts with the Taranna male T4 and King Island male K20, which had overall survival rates of 79 and 95% respectively. In the latter case, four out of five families had 100% survival with the remaining family at 75%. The *E. nitens* female parents appeared to be more consistent in their production of viable hybrid progeny.

Table 5.2 Cross type least squares means for DBHOB at age 2, 3, 4, 6 and 10 years (D2, D3, D4, D6, D10) and Pilodyn penetration at age 6 years (P6) at the West Ridgley site. Units are in millimetres for all traits, standard errors are shown in brackets. Cross types are *E. globulus* intra- and inter-provenance crosses, *E. globulus* CP crosses combined, *E. nitens x globulus* F1 hybrid (split by provenance of *E. globulus* male parent and combined) and *E. nitens*.

Species	Cross type	Trait					
		D2	D3	D4	D6	D10	P6
E. globulus intra-provenance	TT	68.3 (2.0)	110.6 (3.0)	129.2 (3.5)	161.4 (4.4)	192.8 (5.8)	12.8 (0.2)
	KK	73.7 (1.8)	117.7 (2.4)	140.6 (3.1)	181.2 (4.6)	226.9 (7.1)	13.9 (0.2)
E. globulus inter-provenance	KT, TK	71.9 (1.7)	115.0 (2.6)	136.6 (3.0)	176.1 (3.8)	221.2 (4.9)	13.4 (0.2)
E. globulus (combined)	TT, KK, KT, TK	70.9 (1.7)	114.0 (2.5)	134.7 (3.1)	171.4 (4.1)	211.2 (5.3)	13.3 (0.2)
E. nitens x globulus F1 hybrid	NT	62.4 (1.9)	96.2 (3.0)	116.5 (3.4)	165.2 (4.3)	223.4 (5.8)	13.5 (0.2)
	NK	75.4 (2.7)	118.4 (4.2)	146.3 (4.7)	193.4 (5.8)	239.8 (7.1)	13.9 (0.2)
E. nitens x globulus (combined)	NT, NK	66.1 (1.8)	102.5 (2.9)	124.9 (3.4)	174.1 (4.3)	230.2 (5.7)	13.6 (0.2)
E. nitens	NN	77.2 (2.3)	126.4 (3.5)	151.7 (4.0)	190.1 (5.1)	225.3 (6.5)	13.7 (0.2)





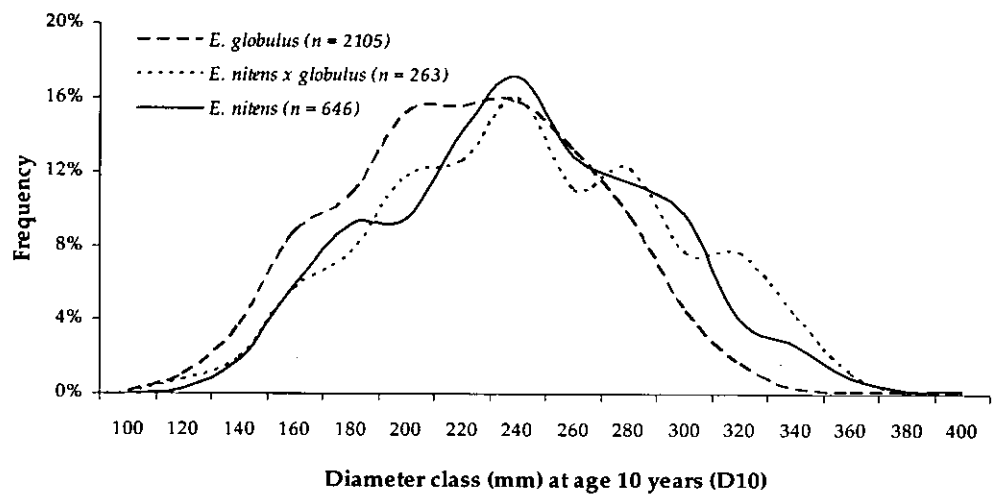


Figure 5.2 Frequency distribution of trees by diameter class at age 2 (D2), 6 (D6) and 10 (D10) years.

Table 5.3: Overall survival expressed as a percentage of the number of trees planted (n) for each cross type at age 6 years as well as the range of survival for each family, female and male within cross type.

Species	Cross Type	n	Overall mean survival (%)	Survival range (%)		
				Family	Female	Male
E. globulus intra-provenance	TT	1076	74	21 - 100	57 - 89	60 - 85
	KK	566	94	70 - 100	90 - 96	88 - 98
E. globulus inter-provenance	KT, TK	1282	88	57 - 100	70 - 100	71 - 98
E. nitens x globulus	NT	467	54	0 - 100	38 - 83	9 - 92
	NK	176	66	18 - 100	56 - 78	45 - 95
E. nitens	NN	701	99	93 - 100	98 - 100	97 - 100

Table 5.4: Mid-parent heterosis for intra-specific hybrid (H_{TK}) and inter-specific hybrids (H_{NT} and H_{NK}), expressed in mm and percent of mean diameter of the mid parent (%). (T = Taranna *E. globulus*, K = King Island *E. globulus*, N = *E. nitens*). The significance of deviation of hybrid mean from mid-parent value was tested with Proc MIXED in SAS where ^{ns} is not significant, * is $P \leq 0.05$, ** is $P \leq 0.01$ and *** $P \leq 0.001$. Positive heterosis for P6 is equivalent to negative heterosis for wood density.

Hybrid type	Trait					
	D2	D3	D4	D6	D10	P6
Intra-specific						
H_{TK}	0.9 (1%)^{ns}	0.8 (1%)^{ns}	1.7 (1%)*	4.8 (3%)*	11.3 (5%)**	0 (0%)^{ns}
Inter-specific						
H_{NT}	-10.4 (-14%)***	-22.3 (-19%)***	-24.0 (-17%)***	-10.6 (-6%)***	14.3 (7%)*	0.2 (2%)^{ns}
H_{NK}	0 (0%)^{ns}	-3.7 (-3%)^{ns}	0.1 (1%)^{ns}	7.7 (4%)^{ns}	13.7 (6%)^{ns}	0.1 (2%)^{ns}

The inter-provenance crosses of *E. globulus* (TK, KT) exhibited mid-parent heterosis for growth (2 to 3%), which increased with age to be significant ($P<0.05$) by age 4 years (Table 5.4). However, the mean growth performance of the inter-provenance cross never exceeded the mean of the better intra-provenance cross, KK (Table 5.2). The poorer growth performance of the TT intra-specific crosses compared with KK is also reflected in the growth performance of the inter-specific hybrids with *E. nitens* at all ages. In the case of the Taranna provenance, the inter-specific F_1 hybrid (NT) showed significant negative mid-parent heterosis for D2, D3, D4 and D6 (Table 5.4), However, at age 10 years, after significant mortality among slower growing individuals (Fig. 5.1 & Fig. 5.2), the mean growth of survivors in this cross type was not significantly different from the better performing cross type NN (Table 5.2). In contrast, heterosis for growth of the inter-specific hybrids of King Island parentage (NK) was not significantly different from the mid-parent value and ranged from 0 to 6% across ages (Table 5.4). It should be noted that the mean diameter of the surviving F_1 hybrids (NT and NK) will be biased upward through reduced competition arising from the high mortality in the hybrid sub-blocks (Table 5.4). This effect will be accentuated by the increased mortality between age 6 and 10 years (Fig 5.2) which would result in relatively higher mean growth performance of survivors (Table 5.2), especially among poorer surviving cross types. This would be due to reduced competition and increased mortality of slower growing trees.

There were large differences in P6 between the two *E. globulus* provenances, with King Island provenance having greater Pilodyn penetration, hence lower density, than the Taranna provenance (Table 5.2). The mean P6 in *E. nitens* (NN) was comparable to the King Island provenance (KK) (Table 5.2). In the inter-provenance (TK and KT) and inter-specific F_1 hybrid crosses involving Taranna *E. globulus* (NT) the mean Pilodyn penetration was intermediate

between the means of the parental populations (Table 5.2) and did not differ significantly from the mid-parent value (Table 5.4). In the inter-specific F_1 hybrid crosses involving King Island *E. globulus* (NK) the mean was slightly below the less dense King Island *E. globulus* (Table 5.2), but was not significantly different from either parent or the mid-parent value (Table 5.4).

Distribution of hybrids

The phenotypic coefficients of variation (CV_P) for growth traits were similar for *E. nitens* and *E. globulus* across all ages, although *E. nitens* CV_P tends to increase with age while in *E. globulus* it remains relatively constant in all cross types. (Table 5.5). The range of variation in the growth of the F_1 hybrids covered the extremes in growth exhibited by the pure species (Fig. 5.2). The phenotypic variation in growth (not shown) of the *E. nitens x globulus* F_1 hybrid population was markedly higher (at least 50%) than either pure species at ages 2 to 6 years contributed by higher components for additive, SCA and residual variances (Table 5.5). Due to mortality among slower growing individuals the *E. nitens x globulus* F_1 hybrid SCA variance reduced to zero and residual variance was more similar to the pure species cross types by age 10 years (Table 5.5). This increased variation for D2 to D6 in the *E. nitens x globulus* F_1 hybrid was not a scale effect, as it was also reflected in a higher CV_P (Table 5.5), and was largely due to the higher level of poor performing, "abnormal" phenotypes. These abnormalities are reflected in the marked tail on the left-hand side of the frequency distribution for D2 in *E. nitens x globulus* F_1 hybrids (Fig. 5.2). It is interesting to note that despite high levels of mortality among these individuals, the tail on the distribution still persists in D6 (Fig. 5.2). At age 10 years, due to mortality among slower growing

individuals, there were no "tails" on the frequency distribution curves (Fig. 5.2) which led to a marked reduction in CV_P (Table 5.5).

Table 5.5 Variance components (± standard error) and coefficients of variation for traits D2, D3, D4, D6, D10 and P6 in *E. globulus*, *E. nitens* and F1 hybrids. Where σ^2_{plot} is plot variance, σ^2_a is additive genetic variance, σ^2_{sca} is SCA variance, σ^2_{res} is residual variance, CV_A is coefficient of additive variation and CV_P is coefficient of phenotypic variation

Trait	Component	*E. globulus*				*E. nitens x globulus*	*E. nitens*
		intra-provenance		inter-provenance	Overall	F1 hybrid	
		TT	KK	TK, KT		NT, NK	NN
D2	σ^2_{plot}	1 (5)	8 (7)	15 (5)	10 (3)	6 (20)	11 (6)
	σ^2_a	49 (24)	20 (13)	36 (14)	33 (11)	326 (156)	33 (21)
	σ^2_{sca}	14 (6)	2 (4)	0	5 (2)	48 (26)	4 (5)
	σ^2_{res}	107 (14)	108 (10)	133 (10)	121 (7)	154 (82)	95 (12)
	CV_A	10%	6%	8%	8%	27%	7%
	CV_P	19%	16%	19%	18%	34%	16%
D3	σ^2_{plot}	7 (10)	6 (11)	22 (11)	17 (6)	15 (41)	16 (11)
	σ^2_a	94 (48)	20 (17)	56 (23)	55 (19)	872 (428)	125(68)
	σ^2_{sca}	34 (12)	5 (7)	0	13 (5)	173 (79)	10 (9)
	σ^2_{res}	211 (18)	204 (17)	320 (20)	262 (13)	418 (223)	164 (37)
	CV_A	9%	4%	7%	6%	29%	9%
	CV_P	17%	13%	17%	16%	37%	14%
D4	σ^2_{plot}	6 (14)	3 (16)	19 (16)	16 (9)	91 (74)	23 (18)
	σ^2_a	132 (60)	37 (29)	98 (38)	91 (29)	1249 (584)	237 (125)
	σ^2_{sca}	22 (12)	9 (11)	0	6 (5)	130(90)	19 (15)
	σ^2_{res}	315 (37)	323 (27)	478 (31)	393 (20)	611 (308)	261 (67)
	CV_A	9%	4%	7%	7%	28%	10%
	CV_P	17%	14%	18%	17%	37%	15%
D6	σ^2_{plot}	19 (30)	0	27 (29)	16 (17)	0	43 (41)
	σ^2_a	210 (93)	85 (62)	187 (81)	164 (54)	1048 (672)	678 (333)
	σ^2_{sca}	15 (18)	16 (21)	12 (18)	18 (10)	325 (171)	31 (28)
	σ^2_{res}	648 (64)	737 (59)	840 (60)	764 (38)	1129 (364)	552 (176)
	CV_A	9%	5%	8%	7%	19%	14%
	CV_P	19%	16%	19%	18%	29%	19%
D10	σ^2_{plot}	70 (53)	0	9 (57)	1 (34)	192 (174)	185 (97)
	σ^2_a	234 (114)	136 (130)	261 (122)	178 (67)	983 (523)	1427 (696)
	σ^2_{sca}	0	88 (63)	16 (33)	37 (21)	0	3 (45)
	σ^2_{res}	1009 (97)	1752 (136)	1673(112)	1543 (68)	1443 (337)	941 (363)
	CV_A	8%	5%	7%	6%	14%	17%
	CV_P	19%	20%	20%	20%	22%	22%
P6	σ^2_{plot}	0	0.02 (0.15)	0.09 (0.10)	0	0	0
	σ^2_a	0.60 (0.27)	0.86 (0.51)	0.58 (0.24)	0.64 (0.20)	0.50 (0.31)	0.33 (0.25)
	σ^2_{sca}	0.02 (0.05)	0.08 (0.10)	0.01 (0.05)	0.02 (0.03)	0.01 (0.09)	0.07 (0.09)
	σ^2_{res}	1.40 (0.17)	1.65 (0.32)	1.62 (0.17)	1.55 (0.02)	2.00 (0.24)	1.89 (0.20)
	CV_A	6%	7%	6%	6%	5%	4%
	CV_P	11%	12%	11%	11%	12%	11%

A scatter plot of family least squares means for P6 and D6 is shown in Figure 5.3. There is considerable overlap of *E. nitens*, *E. globulus* and inter-specific F_1 hybrid family means for both D6 and P6. Some inter-specific F_1 hybrid families appear to have growth that is similar to the best *E. nitens* families, but do not have the high wood density exhibited by the better performing *E. globulus* families. One NK family in particular appears to show outstanding growth compared to all other families in the trial (Fig. 5.3), and very high survival (100%). However it should be noted that the poorer survival among the hybrid families has contributed to a less competitive environment in these sections of the trial overall and may have led to increased diameter growth in surviving individuals. There are 6 inter-specific F_1 hybrid families (5 NT and 1 NK) which exhibit above average growth and below average Pilodyn penetration, compared with many *E. globulus* intra- and inter-provenance families and a few *E. nitens* families, which would be a favourable combination for Kraft pulp production. On the other hand there are a number of NT families which show very poor growth combined with high pilodyn penetration. Most of these families had exhibited high mortality and did not survive to age 10 years (data not presented).

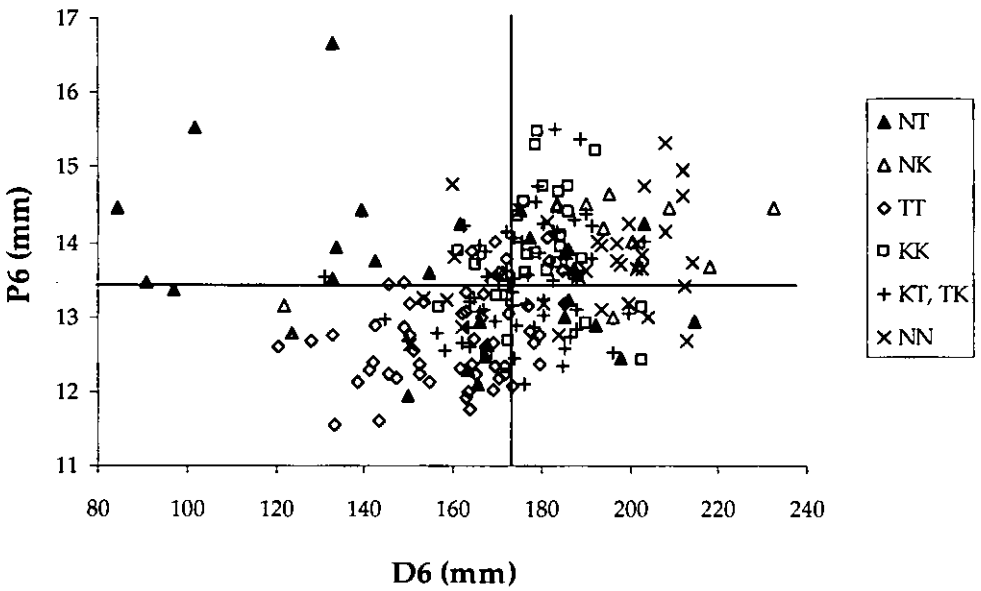


Figure 5.3 Bivariate distribution of family least squares means (adjusted for block effects) for Pilodyn penetration (P6) and diameter as DBHOB (D6) at age 6 years in *E. globulus* intra-provenance crosses in Taranna (TT) and King Island (KK) inter-provenance (intra-specific) hybrids between Taranna and King Island provenances in *E. globulus* (KT), inter-specific F_1 *E. nitens x globulus* hybrids involving *E. globulus* Taranna (NT) and King Island male parents (NK), and intra-specific *E. nitens* crosses from an incomplete half-diallel crossing program (NN) at West Ridley trial site. The overall mean for each trait is shown by the lines perpendicular to each axis.

Genetic parameters

Virtually no significant quantitative genetic variation for growth was detected in the King Island (KK) population, but significant levels of additive genetic variation were detected in the Taranna (TT) population at most ages (Table 5.6). The heritability of growth traits in Taranna intra-provenance crosses (TT) was moderate, at around h^2=0.29±0.12 in D2 decreasing to h^2=0.18±0.08 at age 10. In King Island intra-provenance crosses, the heritability was lower, with h^2=0.15±0.09 in D2 decreasing to h^2=0.07±0.06 at D10 (Table 5.6). The proportion of additive genetic variance expressed in the inter-provenance F_1 is intermediate to that found in either parental population as indicated by the

intermediate heritabilities at all ages (Table 5.6), consistent with intermediate levels of additive genetic variation (CV_A; Table 5.5). While standard errors were high, the dominance variation in the Taranna intra-provenance crosses (TT) at early ages declined to virtually zero by age 6 years (Table 5.6). By contrast, dominance variation (d^2) in the King Island intra-provenance crosses (KK) and the inter-provenances crosses (KT, TK) tended to increase with age but remained low and not statistically different from zero.

Trends over time in heritability of growth differed considerably between the two species and the F_1 hybrid populations (Table 5.6). The trend for *E. globulus* overall was for the heritability of diameter within cross types (within TT, KK, TK and KT) to drop from a moderate value of $h^2 = 0.19 \pm 0.06$ for D2 to $h^2 = 0.10 \pm 0.03$ for D10, these were statistically significant at all ages. Diameter heritabilities for *E. nitens* were initially similar to *E. globulus*, but markedly increased with time from $h^2 = 0.23 \pm 0.13$ for D2 to $h^2 = 0.56 \pm 0.20$ for D10. The diameter heritability for the inter-specific F_1 hybrid was high at all ages and slightly lower than *E. nitens* at ages 6 and 10 (Table 5.6), although differences were not significant. This was a direct reflection of increased additive genetic differences between the parents when in hybrid combination, where CV_A was higher in the inter-specific *E. nitens x globulus* F_1 hybrids than the pure species crosses for ages 2 to 6 years (Table 5.5). At age 10 years CV_A in the *E. nitens x globulus* F_1 hybrids has shown a marked decrease relative to pure species cross types (Table 5.5). In addition the hybrids show a high coefficient of phenotypic variation (CV_P) (Table 5.5) which reflects the wide spread of individuals in the diameter distributions (Fig. 5.2), especially at early ages, in comparison to either of the parental species.

Table 5.6 Heritability (h^2) and dominance ratio (d^2) (and standard errors) in intra- and inter-provenance crosses of *E. globulus*, combined analysis of all crosses in *E. globulus*, inter-species hybrid *E. nitens x globulus* and within species crosses of *E. nitens* for diameter at age 2 (D2), 3 (D3), 4 (D4), 6 (D6), 10 (D10) years and Pilodyn at 6 years (P6).

Species	Cross type		D2	D3	D4	D6	D10	P6
E. globulus	TT	h^2	0.29 (0.12)	0.27 (0.12)	0.28 (0.11)	0.24 (0.09)	0.18 (0.08)	0.30 (0.12)
		d^2	0.33 (0.13)	0.39 (0.14)	0.19 (0.10)	0.07 (0.08)	0	0.03 (0.10)
	KK	h^2	0.15 (0.09)	0.09 (0.07)	0.10 (0.07)	0.10 (0.07)	0.07 (0.06)	0.33 (0.17)
		d^2	0.06 (0.11)	0.08 (0.11)	0.10 (0.12)	0.08 (0.10)	0.18 (0.12)	0.12 (0.16)
	TK, KT	h^2	0.20 (0.07)	0.14 (0.06)	0.16 (0.06)	0.17 (0.07)	0.13 (0.06)	0.25 (0.09)
		d^2	0	0	0	0.05 (0.06)	0.03 (0.07)	0.02 (0.09)
	Overall	h^2	0.19 (0.06)	0.16 (0.05)	0.18 (0.05)	0.17 (0.05)	0.10 (0.03)	0.29 (0.07)
		d^2	0.12 (0.05)	0.14 (0.05)	0.05 (0.04)	0.08 (0.04)	0.08 (0.05)	0.04 (0.05)
E. nitens x globulus		h^2	0.61 (0.22)	0.59 (0.23)	0.60 (0.21)	0.42 (0.23)	0.47 (0.17)	0.20 (0.11)
		d^2	0.36 (0.24)	0.47 (0.27)	0.25 (0.19)	0.52 (0.28)	0	0.02 (0.14)
E. nitens		h^2	0.23 (0.13)	0.40 (0.18)	0.44 (0.18)	0.52 (0.19)	0.56 (0.20)	0.14 (0.10)
		d^2	0.12 (0.12)	0.13 (0.11)	0.14 (0.11)	0.10 (0.09)	0	0.13 (0.15)

Dominance effects (d^2; Table 5.6), estimated from SCA effects among full-sib families (Table 5.5), were significant for *E. globulus* within Taranna crosses (TT) and overall CP crosses for D2 and D3 but not for any other *E. globulus*, *E. nitens* nor the F_1 hybrid traits and cross types. This was partly a reflection of the small number of parents involved in the *E. globulus* intra-specific; *E. nitens x globulus* and *E. nitens* cross types. Dominance effects accounted for between 0 and 39% of the total variance in growth in the pure species crosses, and while relatively stable with age in *E. globulus* overall, tended to decrease with age in *E. nitens* as the heritability increased (Table 5.6). In the inter-specific F_1 hybrids dominance variance for growth was quite high for ages 2 to 6 years but became zero at age 10 years (Table 5.6). This may be a reflection of

mortality among slower growing individuals between ages 6 and 10 years, especially in the NT population (Fig. 5.1).

The heritability of Pilodyn penetration (P6) was higher for *E. globulus* overall (h^2 = 0.29±0.07) than for *E. nitens* (h^2 = 0.14±0.10), with the hybrid intermediate (h^2 = 0.20±0.11) (Table 5.6), although these latter two estimates were not significantly different from zero. Pilodyn penetration exhibited little or no dominance variation in *E. globulus* and the F_1 hybrid population. The d^2 value was comparable to the h^2 estimate in *E. nitens* (Table 5.6), although neither was significantly different from zero

Correlation between growth and Pilodyn

Genetic and phenotypic correlations between D6 and P6 for *E. globulus* overall, *E. nitens* and the F_1 hybrids at this site are shown in Table 5.7. The genetic correlation (r_g) ranged from –0.05 to 0.81, but only the phenotypic correlation in *E. globulus* and the genetic correlation in *E. nitens* was significantly different from zero. However, these correlations were significantly greater than zero with the larger data set used in Chapter 4 for *E. globulus* and *E. nitens*, where the genetic correlations were found to be r_g = 0.40±0.09 and r_g = 0.77±0.12 respectively (Table 4.4). The strong genetic correlation between growth (D6) and Pilodyn penetration (P6) in *E. nitens* suggests that selection for faster growth will lead to a reduction in wood density, which may not be a desirable outcome. There appears to be no genetic correlation between P6 and D6 in the inter-specific *E. nitens x globulus* F_1 hybrid (Table 5.7), which suggests that selection in one trait is unlikely to have any effect on the performance of the other trait in the hybrid.

Table 5.7 Genetic (r_g) and phenotypic (r_p) correlations between growth (D6) and Pilodyn (P6) with standard errors, in brackets, for *E. globulus* (overall), *E. nitens x globulus* and *E. nitens*.

Species	r_g	r_p
E. globulus	0.19 (0.23)	0.19 (0.03)
E. nitens x globulus	-0.05 (0.45)	-0.01 (0.09)
E. nitens	0.81 (0.26)	0.13 (0.07)

Correlation between intra- and inter-specific performance

The estimates of genetic correlation between the inter-specific hybrids and the pure species (i.e. GCA vs. GHA) for growth at all ages and Pilodyn were not significantly different from zero in all cases except for trait D10 in *E. globulus* and *E. nitens* where the correlations were -1.12±0.16 and 0.65± 0.28 respectively (Table 5.8). Nevertheless, there was a trend for the genetic correlation between the *E. nitens* and the *E. nitens x globulus* F_1 hybrid growth performance to increase with time, from 0.35 for D2 to 0.67 for D6. In contrast the correlation between the *E. globulus* and inter-specific *E. nitens x globulus* F_1 hybrid growth performance was much lower for these traits at 0.26 for D2 decreasing to 0.16 for D6 (Table 5.8). The poor correlation with *E. globulus* occurred despite significant levels of additive genetic variation in the pure *E. globulus* overall at all ages. However, the heritabilities for growth in *E. globulus* were lower than those recorded for *E. nitens* (Table 5.6). The magnitude of the inter-specific correlation seems to be related to the expression of additive genetic variance in the pure species parental population, particularly in the case of the increase in heritability for growth with age in *E. nitens* (Table 5.6).

Table 5.8 Genetic correlation (and standard errors) of GCA and GHA for intra- and inter-specific F_1 hybrids. Within *E. globulus* the intra-species hybrid cross types TK and KT were pooled in the analysis as one cross type.

Species	D2	D3	D4	D6	D10	P6
Genetic correlation of GCA with GHA within *E. globulus*						
***E. globulus* (TT)**	0.72 (0.17)	0.79 (0.18)	0.94 (0.12)	0.93 (0.13)	0.97 (0.20)	1.15 (0.06)
***E. globulus* (KK)**	0.72 (0.31)	0.60 (0.43)	0.99 (0.19)	0.91 (0.21)	0.93 (0.27)	0.94 (0.20)
Genetic correlation of GCA with GHA for each parental species with inter-specific F_1 hybrids						
E. globulus	0.26 (0.35)	0.10 (0.40)	0.12 (0.34)	0.16 (0.46)	-1.12 (0.16)	0.60 (0.39)
E. nitens	0.35 (0.50)	0.69 (0.36)	0.45 (0.45)	0.67 (0.46)	0.65 (0.28)	0.68 (0.47)

For Pilodyn penetration (P6), the genetic correlation of parental values in pure was, was positive, but not significantly different from zero in *E. globulus* (r_{gh} = 0.60±0.39) and *E. nitens* (r_{nh} = 0.68±0.47) (Table 5.8). This high pure species vs. hybrid genetic correlation with the *E. globulus* parents for Pilodyn is consistent with the general trend across traits for the correlation (r_{GH}) to become increasingly positive with increasing heritability in the pure species crosses. The *E. nitens* population exhibited no significant additive genetic variation for P6 whereas significant levels were detected in *E. globulus*. However, for the same pure species heritability (*e.g. E. globulus* D2 vs. P6) the pure-hybrid genetic correlation for the Pilodyn penetration was higher than that observed for the growth trait.

In contrast to the poor inter-specific correlation involving *E. globulus*, there was a very high level of genetic correlation between the parental performance in *E. globulus* intra-provenance crosses and the corresponding inter-provenance (intra-specific) hybrids for growth at all ages and P6 (Table 5.8).

Discussion

Inter-specific F_1 hybrid performance

There are many reports of superior performance of inter-specific F_1 hybrids of *Eucalyptus* (de Assis 2000; Potts *et al.* 2000; Verryn 2000; Vigneron and Bouvet 2000; Potts and Dungey 2001), however this does not appear to be the case for the *E. nitens x globulus* F_1 hybrid or for many other hybrid combinations involving *E. nitens* (Tibbits 2000) or *E. globulus* (Lopez *et al.* 2000; Potts *et al.* 2000). In the present case the inter-specific F_1 *E. nitens x globulus* exhibited high incidence of abnormalities at young ages (Potts *et al.* 1992) and high levels of later age mortality. Such inviability of the *E. nitens x globulus* F_1 hybrid has also been reported by Espejo *et al.* (1995). Similarly, inviability is a consistent feature in many other eucalypt hybrid combinations, particularly involving species from the section *Maidenaria,* to which both *E. nitens* and *E. globulus* belong (de Assis 2000; Potts and Dungey 2001). However, even higher levels of inviability have been reported in wider inter-specific crosses and there appears to be a greater tendency towards inviability in hybrids with increasing taxonomic distance (Griffin *et al.* 1988; Griffin *et al.* 2000; Potts and Dungey 2001).

The main genetic causes of inviability of the inter-specific F_1 hybrid include (i) genome disharmony (*e.g.* irregularities during mitosis from major chromosomal differences) and incompatible development cues; (ii) the deleterious, complementary action of one or a few genes; or (iii) cytoplasmic effects (Levin 1978). The deleterious interactions of genes from the same (Orr 1995) or different loci (Lynch 1991) have been implicated in many cases of inviability of F_1 hybrids (Levin 1978, Burke and Arnold 2001). Such genes

that have no deleterious effects within a species, probably accumulate as a by-product of divergence, may cause inviability or sterility in combination with genes from another species. In the present case it was clear that these effects are more prevalent in individual male parents which produced high levels of inviable F_1 progeny across a range of female parents. This result shows that within species, the potential for hybridisation varies considerably and can be parent specific. In other cases, where high mortality occurred, the surviving individuals were of normal phenotype and often relatively vigorous. This effect has been noted in other studies with *Eucalyptus* (de Assis 2000; Perrow (MacRae) and Cotterill 2000; Tibbits 2000), suggesting parents may be heterozygous or polymorphic for factors causing inviability. However, in operational breeding programs it has been demonstrated that effective selection is still possible despite these disadvantages, provided crossing is undertaken at a large scale (Griffin *et al.* 2000).

In this study the mean performance of surviving inter-specific F_1 hybrids of *E. nitens x globulus* for growth (D6) and Pilodyn (P6) traits was generally intermediate, to varying degrees, between the parental intra-provenance crosses. On average, there is a tendency for the surviving inter-specific F_1 hybrids to perform better than the mid-parent value and even the better parent mean at later ages, particularly for crosses involving the King Island *E. globulus*. However, this was rarely statistically significant and was likely to be an artefact of the less competitive environment under which the surviving F_1 hybrids were growing compared with the parental controls, where mortality was relatively minor (especially in *E. nitens*). At age 2 the distribution of diameters in the F_1 hybrids was considerably more skewed than in either parent species, showing a clear excess of trees with very slow growth. With time, abnormal trees died out, leaving a slight tail still evident at age 6 and a more normal distribution for the hybrids by age 10. Therefore there is little

evidence for significant heterosis at the cross type level in the surviving inter-specific hybrids. Similar conclusions are reached from other hybridisation programs involving species from the section *Maidenaria*. In most cases, the inter-specific F_1 hybrid mean was intermediate, or often below, the mid-parent value (Cauvin *et al.* 1987; Potts *et al.* 1992; Dungey *et al.* 1997; Lopez *et al.* 2000; Potts *et al.* 2000; Tibbits 2000). However, this mean response does not exclude the fact that some inter-specific F_1 hybrid individuals or families may be outstanding. Indeed, there was one outstanding inter-specific F_1 hybrid family observed particularly for growth and survival, although its wood density was below average (Fig.5.3). These families were generally parented by King Island *E. globulus*.

The evidence presented here shows that different provenances may exhibit different responses to hybridisation. In this case, the inter-specific F_1 hybrids, using Taranna *E. globulus*, appear to perform worse than those using King Island provenance on average. In this case three out of ten male Taranna parents had very poor survival in the F_1 progeny while all King Island parents showed good survival. This may indicate a provenance difference in hybridisation propensity, but further sampling is required to verify this hypothesis. This was also shown in Chapter 3 for frost tolerance traits, where Taranna provenance *E. globulus* used in inter-specific hybrids, showed poorer mean performance than where King Island parents were used.

A key point in assessing hybrid performance is defining the test environment (Martin 1989; Nikles and Griffin 1992). It has been shown that expression of hybrid superiority is often highly dependent on the environmental effects that may limit performance of one or other of the pure species parents (Nicholas 1987; Potts and Dungey 2001). The most common reason to breed hybrids would appear to be to provide suitable genotypes for planting in areas which

are marginal for the parental species in terms of environmental limitations; *e.g.* frost, drought, waterlogging, wind prone sites, nutritional limitations, pests and diseases (Vigneron and Bouvet 2000; Potts and Dungey 2001; Dungey and Potts 2002]), or to combine economically important traits, (*e.g.* high growth, good form, high wood density, long fibre length) in a single organism. In some cases hybrids are desired to achieve a combination of environmental adaptability and economic trait improvements, as in this study.

In the present case the site was clearly more favourable for *E. nitens* in terms of survival and growth of the survivors and *E. globulus* was not subject to extreme frost damage as it would be at higher altitudes in the same area (Potts *et al.* 2000). Despite this there were still families from all cross types that showed favourable combinations of high growth and low Pilodyn (high wood density). At this site there was no indication of any inter-specific F_1 hybrids with character combinations which could not be found by exploiting the range of genetic variation within the pure species crosses. Potts *et al.* (2000) have shown that with increasing altitude in this area, *E. globulus* growth is severely retarded compared to *E. nitens*. It is unlikely the F_1 hybrid might significantly out-perform the more frost sensitive *E. globulus* in terms of frost resistance (Tibbits *et al.* 1991b and Chapter 3 in this thesis), and given the results it is also unlikely that the F_1 hybrid would outperform or even be equal to *E. nitens* in growth at higher altitudes. The inter-specific F_1 hybrids are likely to show at least slightly improved wood properties, compared to *E. nitens* for eucalypt kraft pulp production(Tibbits *et al.* 1995; present data) if high density *E. globulus* is used as a parent. However, *E. nitens* shows very high growth performance across a wide range of altitudes in this area (Potts *et al.* 2000). It is therefore likely to be less efficient to find genotypes of inter-specific *E. nitens x globulus* F_1 hybrids with comparable growth to *E. nitens* and superior wood

properties, than simply exploiting genetic variation in wood properties within *E. nitens* (Tibbits and Hodge 1998).

There are two issues for consideration in comparing intra-species crosses with inter-species hybrids. Firstly, as demonstrated, there is genetic variation within species at the within and between provenance level which can be exploited. Secondly, heterosis exhibited in inter-provenance crossing may provide just as much gain as can be achieved with inter-specific F_1 hybridisation. This result emphasizes the importance of using the best pure species crosses as controls to assess the comparative value of producing hybrids from both an economic and biological perspective (Eldridge *et al.* 1993).

In this study the inter-specific F_1 hybrids, exhibited high levels of inviability and, at best, the performance of the surviving hybrids was no better than the parental species. In contrast, there is clear evidence for mid-parent heterosis in growth for inter-provenance crosses of *E. globulus,* which appeared to be increasing with age. Evidence for heterosis in inter-provenance crossing has been reported in several forest tree species (Schmidtling and Nelson 1996; Harfouche *et al.* 2000; Johnston *et al.* 2001). This is possibly a reflection of removal of mild inbreeding effects (Hardner *et al.* 1996) within provenances. Nevertheless, this heterosis did not result in progeny of inter-provenance crosses outperforming progeny of the faster growing intra-provenance crosses from King Island in this study, although on average, the wood density would be improved.

Genetic parameters

Intra-species crosses

In *E. globulus* intra-provenance crosses it appears that King Island provenance has lower levels of additive genetic variance for growth traits than Taranna provenance as indicated through the coefficient of additive variance (CV_A). Current models used for genetic evaluation assume homogeneous additive genetic variance across populations within a species (Dutkowski *et al.* 1997). The differences in heritability observed in this study provide the first indications that the level of additive genetic variance may differ between populations of *E. globulus,* although larger sample sizes are required to verify this result. Consistent with the expectations from quantitative genetic models (Lo *et al.* 1993; Falconer and MacKay 1996), the intra-specific hybrid exhibits intermediate heritabilities and intermediate levels of additive genetic variance (Tables 5.5 and 5.6). Taranna provenance has moderate heritability for growth, which decreases slightly with time, whereas the estimated dominance variation is less and relatively constant. Later age growth in King Island has low heritability and stable low levels of dominance indicating very low levels of genetic variation for diameter growth in this provenance. The inter-provenance crosses demonstrate a relatively constant level of dominance while heritability decreases with time. When viewed overall, *E. globulus* shows decreasing heritability for growth over time and low but relatively stable level of dominance variation (Table 5.6). Other reports on age trends of genetic variance components in forest trees show a pattern of decreasing dominance with age for height growth traits (Kremer 1981; Balocchi *et al.* 1993; Dieters *et al.* 1995; King *et al.* 1998; Harfouche and Kremer 2000). The generally lower dominance effects in the inter-provenance crosses are

consistent with their expression in intra-provenance crossing being due to inbreeding and deleterious gene effects. The combined analysis across sites in Chapter 4 yielded low heritability and no significant dominance for growth traits, although the expression of dominance differed between sites (Table 4.6).

Hardner and Tibbits (1998) found decreasing heritabilities and greater levels of dominance with increasing age for *E. nitens* diameter growth traits, whereas in this case, heritability of diameter growth increased and dominance decreased with age. In the former case standard errors were much higher, in comparison to the estimated genetic parameters. Across-site analysis in Chapter 4 shows no dominance and increasing heritability with age for *E. nitens* CP material (Table 4.6).

Intra-specific versus inter-specific F_1 hybrids

There are reports of genetic parameters for inter-specific hybrid populations estimated for breeding and deployment purposes (Bouvet and Vigneron 1995; 1996b; Dungey *et al.* 2000a; Gwaze *et al.* 2000; Rezende and de Resende 2000). However, the general lack of pure species controls means that there is little information on whether hybrid populations behave similarly to pure species populations and conform to current quantitative genetic models.

In this study the performance (as discussed previously) and genetic parameters clearly indicate the genetic behaviour of the inter-specific F_1 hybrid population differs markedly from the inter- and intra-provenance crosses within species. This is reflected by the fact that the coefficients of additive genetic variation and heritabilities for growth are inflated compared to the pure species controls with common parentage and other reports from the literature (see Chapter 4). However, this was not the case for the inter-provenance F_1 hybrids where the level of additive genetic variation in the

intra-specific F_1 hybrid population are intermediate between that exhibited in the two intra-provenance populations, which is expected under an infinitesimal gene model (Lo *et al.* 1993). Furthermore, the additive genetic effects estimated for growth in the pure species populations (GCA; particularly for *E. globulus*) do not correlate with additive genetic effects estimated from the inter-specific hybrid population (GHA), whereas they do in the intra-specific hybrids. This result argues for completely different expression of genetic variation in the hybrid population, at least for genes affecting growth.

In contrast to growth, more typical quantitative genetic behaviour of the hybrid population was observed for Pilodyn penetration in the present study. The evidence to support this includes (i) levels of additive variation in the hybrid population were comparable to those in the pure species population and (ii) the correlation of genetic effects expressed in the *E. globulus* and *E. nitens* pure species crosses is positively correlated with that expressed in the hybrid population, although these correlations had high standard errors, which brings into question their statistical significance. In this case, selection of *E. globulus* and *E. nitens* parents with high wood density would be expected to result in hybrids with higher than average wood density, however due to the large standard error associated with the genetic correlation estimates this hypotheses requires further testing.

The correlation between pure and hybrid performance has been examined in a number of forest species (Nikles and Newton 1991; Powell 1993; Dieters and Dungey 2000; Dungey and Nikles 2000; Schneck and Langner 2000; Tibbits 2000; Vigneron and Bouvet 2000), including eucalypts, but sample size in virtually all cases is small. Specifically, what is important to breeders is the correlation of the general combining ability (GCA) of parents in pure species

to their general hybridising ability (GHA). This correlation varies between species and traits. Poor GCA/GHA correlations occur for growth and frost (Chapter 3) traits in this study, but the correlation is better for other traits such as Pilodyn penetration (this study) and disease resistance (Dungey *et al.* 1997). In the latter case, the GHA correlations were not significantly different from zero, but they were consistently positive. Although not estimated in the same trial or statistically tested, previously derived pure *E. urophylla* breeding values for growth were well correlated with GHA values derived from *E. urophylla x grandis* (0.63) and *E. urophylla x pellita* (0.83) F_1 factorials grown in Congo (Vigneron and Bouvet 2000). As expected, a strong correlation between the *E. urophylla* GHA values estimated from the two different hybrid combinations (0.64) was also reported. In South Africa, Verryn (2000) also reports a positive, but insignificant correlation (0.65) between previously estimated GCA of *E. grandis* parents and their GHA in *E. grandis x saligna* F_1 hybrid trial. Powell and Nikles (1996) found correlation for GCA and GHA in hybrids of southern pines to be approximately $r = 0.70$ for diameter and height while the correlation for stem straightness was not significantly different from zero.

The poor genetic correlation between pure species and hybrid performance, particularly in the *E. globulus* parents, for growth observed in the present study could arise if (i) different genes are the determinants of a trait, such as growth, in the hybrid population compared to those in one or other parental population such as genes determining disease resistance (Vigneron and Bouvet 2000), (ii) hybrid combinations are influenced more by non-additive than additive genetic effects (Dieters and Dungey 2000), or (iii) other genetic factors such as chromosomal structural differences impact on hybrid performance. This result is consistent with the theoretical treatment (Gordon 1999), showing that hybrid populations are profoundly different from a

random mating population, and that the estimates of additive genetic variance in pure and hybrid populations are not theoretically comparable. Bouvet and Vigneron (1996b) similarly note that results concerning additive and dominance variance in hybrid populations must be treated with caution as (i) the hypothesis of a unique panmictic population is not verified as the parent trees belong to different provenances (ii) epistatic effects can not usually be estimated and are not included in the model. These epistatic effects are included into additive and dominance effects and may lead to an overestimate of these effects if large (Falconer and MacKay 1996). However, while epistatic effects are likely to be small in pure species populations (Stonecypher and McCullough 1981; Gallais 1988a; 1988b), this may not be the case in interspecific hybrid populations. Indeed, epistatic interactions between the divergent genomes of the different species may well override the additive genetic effects within pure species, which are normally consistently expressed in intra- and inter-provenance crossing. Most of these non-additive inter-specific interactions appear to be deleterious as some parents show high levels of inviability and poor growth in inter-specific hybrid combination, which cannot be predicted from knowledge of classical quantitative genetic models.

The model is not correct, in that differences between families in hybrids may be due to deleterious epistatic interactions or segregation of major genes, which is not catered for in classical quantitative genetic theory. The high level of abnormalities observed in the nursery and at a young age in the hybrids (Dungey 1991; Potts *et al.* 1992), suggests that genetic mechanisms other than those expected under an infinitesimal gene effect model, may be operating in the hybrid population. These abnormalities were either lethal or have reduced the growth to such an extent that the individuals concerned were not able to survive competition from neighbouring trees. However the problem was not confined only to major abnormalities. Bouvet and Vigneron (1996b)

reported a level of abnormalities around 10% for *E. urophylla x grandis*. These problems also affected the analysis in the present study. For example, the average family performance was reduced and family variance was considerably inflated at early stages in the inter-specific hybrids. Once the less vigorous and deformed trees died, there was a corresponding effect on family performance through decreased competition at later ages, as a result of gaps amongst the hybrid plots. For these effects to result in such a marked increase in heritability for growth they occurred only within a few families, and never in others, causing heritability to decrease with time. This suggests a strong genetic basis to inter-specific hybrid performance, as suggested by Baril *et al.* (1997a; 1997b) in an *E. urophylla x E. grandis* hybrid.

In generating individuals from more than one breed, (population or species) we aim to exploit favourable dominance deviations. However, there is a risk that favourable epistatic relationships, which have been established within the breeds, will be broken down (Kinghorn 1980). In addition, there is evidence that epistatic variance can be converted into additive variance following a founder event (Goodnight 1988) or crosses between distantly related individuals, breed or species (Lynch 1991). This non-linear response to the degree of outcrossing suggests there is a fundamental change in the predominant gene interactions as mates become more and more distantly related (Lynch 1991).

Implication for making choices in breeding and deployment

The implications of varying levels of GHA compared with GCA for different traits or parental species has implications for hybrid breeding strategy. In the case presented here it appears that wood density in *E. globulus* is a trait that behaves as predicted according to classical quantitative genetic theory in

intra-/inter-provenance and inter-specific crosses. This reflects the strong genetic control of this trait. However, it becomes clear from the result, that as the level of genetic variance is reduced, so too is the ability of a species to express its genetic merit in a predictable manner in a hybrid combination.

The result illustrates the importance of accounting for provenance or race effects for growth and Pilodyn penetration (wood density). Despite the fact that *E. globulus* produces a denser wood than *E. nitens*, the differences depend on the specific *E. globulus* races compared. In our study, trees from King Island were less dense (higher Pilodyn) than the *E. nitens* material from Toorongo, whereas trees from Taranna *E. globulus* were about 10% denser. Trees from King Island grew significantly better (about 10% also) than Taranna, so the overall merit of each cross type depends on the relative importance given to volume and wood density in the breeding objective.

In eucalypts, the relative value given to wood density in breeding for Kraft pulp production is marginally higher than for volume, but differences are small, and depend on the cost structure, discount rate and selection criteria (Greaves *et al.* 1997a). Therefore the use of *E. nitens x globulus* F_1 hybrid would need to show significantly improved wood quality and growth compared to *E. nitens* to be useful in Tasmania. The indications are that there is no significant genetic correlation between growth and density in the inter-specific *E. nitens x globulus* F_1 hybrid.

Proposed and commercially used strategies for breeding eucalypt hybrids have been reviewed (Shelbourne 2000; Verryn 2000). The two main strategies that have historically dominated the hybrid breeding literature are reciprocal recurrent selection (RRS) (Comstock *et al.* 1949; Hyun 1976) and recurrent selection (RS). If the performance of the hybrids can be predicted easily from the performance of parents in the pure-breds, then RS is the simplest

alternative. However, as noted by Potts and Dungey (2001), RRS has obvious advantages where tests of both pure species are not possible (Vigneron and Bouvet 2000) or if there is a poor correlation between performance of parents in hybrid and pure species combinations. A RRS strategy, which is the most appropriate given the lack of parental predictability (low correlation of GCA with GHA) would require the removal of parents which produce inter-species F_1 hybrid families with a propensity to produce abnormal phenotypes with low vigour and/or poor form. This strategy adds significant cost and time to a breeding program, which limits the achievement of gain per unit time. However, as classical quantitative genetic theory does not appear to explain the behaviour of inter-specific hybrids there is doubt as to whether the normal measures of inheritance such as narrow sense heritability can be interpreted in the same way as applied to intra-specific crosses (Gordon 1999) and genetic gains would be more difficult to predict.

Nikles and Griffin (1992) pointed out that hybrids are used either because of heterosis or complementarity between species. In the present case the hybrid demonstrates little heterosis and limited complementarity. It must be considered that the hybrid would be required to demonstrate a significant advantage over the parent species to warrant the breeding and propagation effort required to produce a deployment population. *E. nitens x globulus* is difficult to propagate by vegetative means, such as cuttings or tissue culture. Given the low level of heterosis and unreliability of prediction of hybrid performance demonstrated here, it would be dangerous to adopt a seed based strategy at least for the first generation hybrids. Some gain may be possible through initial screening of parents already established in pure species breeding arboreta for traits such as wood density and superior rooting ability. However, as has been demonstrated here there will be significant progeny

testing required to determine the worth of particular parents within species for generating inter-specific hybrids.

In this study the hybrids did not show any clear advantage over either of the parental species and more progress could probably be made by concentrating effort on improvement within species rather than through a hybrid program. For example, there is no evidence that the hybrid combines high wood density of *E. globulus* with fast growth of *E. nitens* to an extent where there is a significant difference between the hybrid and *E. nitens* and also in frost resistance the hybrid is not significantly different from the least resistant parent (*E. globulus*).

There is evidence in this study that specific hybrid families are produced that outperform most of the pure species families for one or other of the traits examined. This result confirms that it is possible to "discover" such families and individuals, which could be exploited. However, this study has shown that there is no reliable quantitative genetic method of predicting which parents will produce outstanding inter-specific F_1 hybrid combinations. The hybrid combination of *E. nitens x globulus* therefore, does not appear to demonstrate any clear advantages to the tree breeder due to the unpredictable performance of progeny and difficulty with production and propagation.

The present study demonstrates the importance of understanding the fundamental genetic parameters behind the performance of hybrids, which are commonly used in forestry. There are many examples of successful hybrid combinations of eucalypts used commercially around the world. These have often been exploited by way of vegetative propagation. Despite the size of many of these commercial hybrid programs, very little work has been done on the basic genetic parameters of the hybrids or their parental species. Consequently, there is little information available that allows evaluation of

merit for potential parents or the prediction of gain in inter-specific hybrid breeding programs. This does not bode well for future genetic improvement of these F_1 hybrid taxon.

Chapter 6: Conclusions

The test of suitability of the two species *E. globulus* and *E. nitens* for use in hybrid combination has been thoroughly examined in this thesis. In addition the use of genetic parameter estimates derived from open-pollinated (OP) material has been examined in direct comparison with control-pollination (CP) using the same parents. The conclusions from this study are:

1. OP estimates of genetic parameters can be unreliable for many traits, but particularly growth traits.

   The accuracy of selection and prediction of genetic gains in a species is determined by the confidence that can be placed in genetic parameter estimates and the estimation of breeding values. This study, combined with work on other eucalypt species, has shown that OP progenies from native stands do not provide accurate genetic parameter or breeding value estimates for growth or frost resistance, especially in *E. globulus*.

   The extent of the adverse additive genetic correlation between Pilodyn and growth traits may have been under-estimated from OP progeny (Chapter 4). This has serious implications for breeding strategies that seek to improve wood density and growth simultaneously.

   To date major breeding programs in Australia have relied on genetic correlations derived from OP progeny tests. OP estimates in *E. globulus* and to a lesser extent in *E. nitens* appear to be confounded by differential levels of inbreeding at the tree and population levels within the native stands.

OP progeny trials appear to have under-estimated the importance of additive genetic x environment interactions in *E. globulus* (Chapter 4). This is important as the level of genotype x environment interaction determines whether single or multiple breeding populations are required for a plantation program that may cover a number of regions and environments.

2. There is evidence for differences in the levels of genetic variance within races of *E. globulus* that may need to be accounted for in intra- and inter-specific hybridization programs.

   It is clear from this study that there were different levels of additive genetic variance and total phenotypic variance for frost (Chapter 3), growth and wood density (Pilodyn) traits (Chapter 4) within the populations of *E. globulus* (Taranna and King Island). Current genetic evaluation models used in breeding programs assume these variances are homogenous across races.

3. Inter-provenance hybrid populations of *E. globulus* appear to behave as expected from quantitative genetic theory. These populations exhibited intermediate levels of additive variance and a strong correlation between parental performance in intra- and inter-provenance hybrid populations.

   There was good correlation of GCA values derived from intra- and inter-provenance crosses within *E. globulus* (Chapter 4 and 5). This was despite the differences in levels of additive genetic variance between provenances (Chapter 4). These differences in variability and heritability estimates were intermediate in the inter-provenance hybrid population. However, the generality of this result needs to be examined further in a more comprehensive inter-provenance crossing program.

4. There is evidence that genetic differentiation in *E. globulus* and *E. nitens* affects hybrid performance, particularly viability and production of abnormal phenotypes which, at the moment, cannot be predicted prior to crosses being undertaken.

   The production of the inter-specific hybrids has been marked by low seed yields (Chapter 2), high frequency of abnormal and inviable phenotypes that became evident in the nursery (Potts *et al.* 1992) and persisted in the field until at least 6 years after planting (Chapter 5).

5. There is no significant correlation of GCA with GHA, particularly in *E. globulus,* therefore the performance of inter-specific F_1 hybrids cannot be predicted from the parental breeding values derived from intra-specific performance.

   The implications of varying association of GHA and GCA for different traits or parental species have implications for hybrid breeding strategy. In the case presented in this study it appears that Pilodyn (an indirect measure of wood density) is a trait that behaves according to classical quantitative genetic theory at all levels of intra- and inter-specific crosses (Chapter 4 and 5). This reflects the very strong genetic control of this trait. However, it becomes clear that as the level of additive genetic variance is reduced, so too is the ability of a species to express its genetic merit in a predictable manner in an inter-specific hybrid combination (Chapter 3 and 5). This is particularly problematic for growth traits.

6. There is evidence that most traits in the inter-specific hybrid are intermediate compared to the intra-specific performance or deviate to the performance of one or other of the parental species. There is, at best, weak

mid-parent heterosis for some traits such as later-age growth, but no evidence of average performance that exceeds that of either parent for any trait examined.

In this study the inter-specific F_1 hybrid did not show any significant advantage over either of the parental species. In the case of frost there is strong evidence that the performance of *E. nitens x globulus* is closer in performance to the more frost-sensitive *E. globulus* (Tibbits *et al.* 1991b and Chapter 3 in this thesis). One of the most important reasons for producing the hybrid was to try and increase the range of "*globulus* like" material by improving the frost resistance in the hybrid compared to *E. globulus*. In other traits such as growth and Pilodyn the evidence is that the hybrids do not show any combination of these traits which could not be found within the parental species even though there was a couple of inter-specific F_1 hybrid families which were ranked among the top families, particularly for growth (Chapter 5). It has been pointed out that classical quantitative genetic theory does not appear to adequately explain the performance of inter-specific hybrids and the normal measures of inheritance may not be able to be applied in the same way as for intra-specific crosses.

7. Implications for breeding and deployment

It has been shown that inter-specific hybrids are used either because of heterosis or complementarity between species (Chapter 1). In this case the inter-specific F_1 hybrid demonstrates little heterosis and limited complementarity for the traits examined on one site (Chapter 5). It must be considered that the hybrid would be required to demonstrate a significant advantage over the parent species to warrant the breeding and propagation effort required to produce a deployment population.

A Reciprocal Recurrent Selection strategy, which is the most appropriate given the lack of predictability (low correlation of GCA with GHA), requires the removal of inter-species F_1 hybrid families and pure parents with a propensity to produce abnormal phenotypes with low vigour and/or poor form. It requires progeny testing to determine which parental combinations produce outstanding families. Exploitation of outstanding individuals within families for clonal propagation requires that these individuals can be identified and that they can be propagated by vegetative means.

A breeding concept that incorporates separate improvement of the parental species and then crossing outstanding individuals to produce F1 hybrids may have some merit if undertaken on a large scale and utilises parents already established in arboreta. However, this study does not provide enough information to test the validity of this concept. Exploitation of such hybrids would still be limited by the ability to propagate them in large numbers.

E. nitens and *E. globulus* are difficult to propagate by vegetative means, such as cuttings or tissue culture and there is no evidence to suggest that the inter-specific F_1 hybrid is any different. Therefore, breeding strategies must incorporate the use of seed as a means of propagation or screening of potential F_1 hybrids for vegetative propagation ability. The latter would be more favoured, as current knowledge suggests the small-flowered *E. nitens* must be used as a maternal parent. Therefore, a seed based strategy for inter-specific *E. nitens x globulus* F_1 hybrid production would be impractical, given low capsule survival and seed yields (Chapter 2). In the latter case, the use of vegetative propagation

as a further screening trait may have an impact on the ability to exploit the most favourable combinations.

The results of this study suggest that more progress could be made by concentrating effort on genetic improvement of a trait or combination of traits through breeding within species rather than through a hybrid breeding program for the traits and species combinations examined here.

In the case of intra-specific hybridisation within *E. globulus* it has been shown, that despite differences in performance and levels of genetic variation within provenances (or races) with wide geographic separation, the inter-provenance performance can be reliably predicted from intra-provenance cross performance using classical quantitative genetic theory (Chapter 4 and 5).

This is the most comprehensive examination of hybridisation in *Eucalyptus* where material of known genetic background has been utilised. The study has demonstrated that a thorough understanding of the fundamental genetic parameters behind the performance of hybrids is required. The hybrid combination used in this study does not demonstrate any advantages due to the unpredictable performance of progeny and the difficulty with propagation (examined by others). There are many successful examples of hybrids being commercially exploited around the world. However, none of these programs to date, have been based on sound knowledge of the genetic parameters of the parental species and only a few have been based on a thorough investigation on genetic parameters within the hybrids themselves (Vigneron and Bouvet 2000). Consequently there is little information available that allows evaluation of merit for potential parents or the prediction of gain in breeding programs. Exploitation of hybrids will continue to be confined to adventitious discovery

of unique phenotypes combined with high multiplication rates through vegetative propagation while this situation continues. This does not bode well for future genetic improvement of parental species to allow production of F_1 hybrid taxon or understanding of the genetic basis behind their perceived superior performance.

References

Allard, R.W. (1960). 'Principles of Plant Breeding'. (Wiley and Sons, New York).

Almeida, M.H., Pereira, H., Miranda, I. and Tome, M. (1995). Provenance trials of *Eucalyptus globulus* Labill. in Portugal. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 195-198. (CRC for Temperate Hardwood Forestry, Hobart).

Anon. (2001). Developing the Eucalypt of the Future. *Proc. of* IUFRO International Symposium. Valdivia, Chile, 10 - 15 Sep. 2001. (Eds. S. Barros and R. Ipinzà) . (INFOR, Valdivia; CD-ROM).

Araújo, J.A., Sousa, R., Lemos, L. and Borralho, N.M.G. (1996). Estimates of genetic parameters and prediction of breeding values for growth in *Eucalyptus globulus* combining clonal and full-sib progeny information. *Silvae Genetica* 45, 223-226.

Arnold, R., Carmona, J.C. and Balocchi, C. (1991). Programa de propagacion clonal para *Eucalyptus globulus* en Chile. *Bosque* 12, 3-9.

Ashton, D.H. (1958). The ecology of *Eucalyptus regnans* F. Muell.: The species and its frost resistance. *Australian Journal of Botany* 6, 154-76.

Awe, J.O. and Shepherd, K.R. (1975). Provenance variation in frost resistance of *Eucalyptus camaldulensis* Dehn. *Australian Forestry* 35, 26-33.

Balocchi, C.E., Bridgwater, F.E., Zobel, B.J. and Jahromi, S. (1993). Age trends in genetic parameters for tree height in a non-selected population of Loblolly pine. *Forest Science* 39, 231-251.

Bamber, R.K. (1985). The wood anatomy of eucalypts and papermaking. *Appita* 38, 210-216.

Baradat, P., Labbé, T. and Bouvet, J.M. (1994). Conception d'index por la sélection réciproque récurrente: aspectes génétiques, statistiques et informatiques. *In* 'Traitements Statistiques des Essais de Séletion. Stratégies d'Amélioration des Plantes Pérennes'. *Proc. of* Actes du Séminaire de Biométrie et Génétique Quantitative. Montpellier, France, 12-14 Sep. 1994 pp. 101-150. (CIRAD, France).

Baril, C.P., Verhaegen, D., Vigneron, P., Bouvet, J.M. and Kremer, A. (1997a). Structure of the specific combining ability between two species of *Eucalyptus*. 1. RAPD data. *Theoretical & Applied Genetics* 94, 796-803.

Baril, C.P., Verhaegen, D., Vigneron, P., Bouvet, J.M. and Kremer, A. (1997b). Structure of the specific combining ability between two species of *Eucalyptus*. II. A clustering approach and a multiplicative model. *Theoretical & Applied Genetics* 94, 804-809.

Beadle, C.L., Turnbull, C.R.A. and Dean, G.H. (1996). Environmental effects on growth and kraft pulp yield *Eucalyptus globulus* and *E. nitens*. *Appita* 49, 239-242.

Becker, W.A. (1984). 'Manual of Quantitative Genetics'. 4th Ed. (Academic Enterprises, Pullman, Washington).

Bertolucci, F.L.G., Demuner, B.J., Garcia, S.L.R. and Ikemori, Y.K. (1995). Increasing fibre yield and quality at Aracruz. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 31-34. (CRC for Temperate Hardwood Forestry, Hobart).

Blake, T.J. and Bevilacqua, E. (1995). Early selection of *Eucalyptus grandis* clones in central Brazil. *Journal of Tropical Forest Science* 8, 33-43.

Borralho, N.M.G. (1992). 'Genetic Improvement of *Eucalyptus globulus* Labill. ssp. *globulus* for pulp production.' Unpublished PhD Thesis, Oxford University.

Borralho, N.M.G. (1994). Heterogeneous selfing rates and dominance effects in estimating heritabilities from open pollinated progeny. *Canadian Journal of Forest Research* 24, 1079-1082.

Borralho, N.M.G. (1997). Seed orchards or cuttings: which is the best? *In* 'Silviculture and Improvement of Eucalypts'. *Proc. of* IUFRO/EMBRAPA Conf. Salvador, Bahia, Brazil, 24-29 Aug. 1997. (Eds. A.R. Higa, E. Schaitza and S. Gaiad) pp. 330-336. (EMBRAPA, Colombo).

Borralho, N.M.G., Cotterill, P.P. and Kanowski, P.J. (1992a). Genetic control of growth of *Eucalyptus globulus* in Portugal. II. Efficiencies of early selection. *Silvae Genetica* 41, 70-77.

Borralho, N.M.G., Cotterill, P.P. and Kanowski, P.J. (1992b). Genetic parameters and gains expected from selection for dry weight in *Eucalyptus globulus* ssp. *globulus* in Portugal. *Forest Science* 38, 80-94.

Borralho, N.M.G., Jarvis, S.F. and Potts, B.M. (1995). Multivariate restricted maximum likelihood estimation of genetic parameters for diameter at 3 to 5 years in the *Eucalyptus globulus* base population in Australia. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 230-232. (CRC for Temperate Hardwood Forestry, Hobart).

Borralho, N.M.G., Kanowski, P.J. and Cotterill, P.P. (1992c). Genetic control of growth of *Eucalyptus globulus* in Portugal I. Genetic and phenotypic parameters. *Silvae Genetica* 41, 39-45.

Borralho, N.M.G. and Potts, B.M. (1996). Accounting for native stand characteristics in genetic evaluations of open pollinated progeny from *Eucalyptus globulus* base population. *New Forests* 11, 53-64.

Borralho, N.M.G. and Wilson, P.J. (1994). Inheritance of initial survival and rooting ability in *Eucalyptus globulus* Labill. ssp. *globulus* stem cuttings. *Silvae Genetica* 43, 238-242.

Bouvet, J.M. and Bailleres, H. (1995). Expression of some growth and wood property traits among *Eucalyptus urophylla* x *grandis* clones in Congo. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 89-92. (CRC for Temperate Hardwood Forestry, Hobart).

Bouvet, J.M., Couteau, N. and Vigneron, P. (1992). Factorial plans analyses used in eucalypts reciprocal recurrent selection in Congo. *In* 'Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species'. *Proc. of* ACTES Conference. Bodeaux, 14-18, Sept. 1992 pp. 317-326. (AFOCEL, France).

Bouvet, J.M. and Vigneron, P. (1995). Age trends in variances and heritabilities in *Eucalyptus* factorial mating designs. *Silvae Genetica* 44, 206-216.

Bouvet, J.M. and Vigneron, P. (1996a). Effect of spacing on heritability of *Eucalyptus* clone and family populations. *In* 'Tree Improvement for Sustainable Tropical Forestry'. *Proc. of* QFRI-IUFRO Conf. Caloundra, Queensland, 27 Oct.-1 Nov. 1996. (Eds. M.J. Dieters, A.C. Matheson, D.G. Nikles, C.E. Harwood and S.M. Walker) pp. 185-186. (QFRI, Brisbane).

Bouvet, J.M. and Vigneron, P. (1996b). Genetic structure of *Eucalyptus urophylla* x *E. grandis* population in the reciprocal recurrent selection scheme in the Congo. *In* 'Tree Improvement for Sustainable Tropical Forestry'. *Proc. of* QFRI-IUFRO Conf. Caloundra, Queensland, 27 Oct.-1 Nov. 1996. (Eds. M.J. Dieters, A.C. Matheson, D.G. Nikles, C.E. Harwood and S.M. Walker) pp. 127-132. (QFRI, Brisbane).

Bouvet, J.M. and Vigneron, P. (1996c). Variance structure in *Eucalyptus* hybrid populations. *Silvae Genetica* 45, 171-177.

Bowden, A.G. (1964). Hybridisation in *Eucalyptus*. *The Indian Forester* 90, 581-586.

Brooker, M.I.H. (2000). A new classification of the genus *Eucalyptus* L'Her. (Myrtaceae). *Australian Systematic Botany* 13, 79-148.

Brown, A.G. (1972). The role of the hybrid in forest tree breeding. *IUFRO Genetics-Sabrao Joint Symposia, Tokyo* C-1, 1-12.

Brown, C. (2000). 'The global outlook for future wood supply from forest plantations.' Global Forest Products Outlook Study Working Paper Series No. GFPOS/WP/03. (FAO Forest Policy and Planning Division, Rome).

Brune, A. and Zobel, B.J. (1981). Genetic base populations, gene pools and breeding populations for *Eucalyptus* in Brazil. *Silvae Genetica* 30, 146-149.

Burdon, R.D. (1977). Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. *Silvae Genetica* 26, 168-175.

Burgess, I.P., Williams, E.R., Bell, J.C., Harwood, C.E. and Owen, J.V. (1996). The effect of outcrossing rate on the growth of selected families of *Eucalyptus grandis*. *Silvae Genetica* 45, 97-101.

Burgess, L.P. (1983). Tolerance of two years old *Eucalyptus grandis* and *Eucalyptus saligna* to winter frost in Canberra, Australian Capital Territory. *In* 'Colloque international sur les *Eucalyptus* résistants au Froid'. *Proc. of* IUFRO/AFOCEL Conf. Bordeaux, France, September 1983 pp. 358-366. (AFOCEL, Paris).

Burke, J.M. and Arnold, M.L. (2001). Genetics and the fitness of hybrids. *Annual Review of Genetics* 35, 31-52.

Campinhos, E. (1980). More wood of better quality through intensive silviculture with rapid-growth improved Brazilian *Eucalyptus*. *Tappi* 63, 145-147.

Campinhos, E. (1999). Sustainable plantations of high-yield shape *Eucalyptus* trees for production of fiber: the Aracruz case. *New Forests* 17, 129-143.

Campinhos, E. and Ikemori, Y.K. (1977). Tree improvement program of *Eucalyptus* spp.: preliminary results. *In* 'Third World Consultation on Forest Tree Breeding'. *Proc. of* FAO Conf. Canberra, Australia, 21-26 Mar. 1977 pp. 717-738. (CSIRO Canberra).

Campinhos, E. and Ikemori, Y.K. (1984). Transcript of lecture by prize winners. *In* 'The New Eucalypt Forest'. *Proc. of* The Marcus Wallenberg Foundation Symposia. Falou, Sweden, 14 Sep. 1984. (Marcus Wallenberg Foundation, Sweden).

Campinhos, E. and Ikemori, Y.K. (1989). Selection and management of the basic population *Eucalyptus grandis* and *E. urophylla* established at Aracruz for the long term breeding programme. *In* 'Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry'. *Proc. of* IUFRO Conf. S2.02. -08. Pattaya, Thailand, Nov. 1988. (Eds. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 169-175. (Oxford Forestry Institute, Oxford, United Kingdom; Winrock Interantional, Arlington, Virginia, USA).

Cannon, P.G. and Shelbourne, C.J.A. (1991). The New Zealand eucalypt breeding programme. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schönau) pp. 198-208. (Southern African Institute of Forestry, Pretoria).

Cauvin, B. (1983). *Eucalyptus* hybridation controlée. Premiers resultats. *In* 'Annales de Recherches Sylvicoles'. Vol. 1982 pp. 85-117. (AFOCEL, Paris).

Cauvin, B. (1988). *Eucalyptus:* Les test de résistance au froid. *In* 'Annales de Recherches Sylvicoles'. Vol. 1987 pp. 161-195. (AFOCEL, Paris).

Cauvin, B., Potts, B.M. and Najar, M. (1993). Estimation de paramètres genetiques de resistance au froid et de croissance pour *Eucalyptus gunnii*. *In* 'Annales de Recherches Sylvicoles'. Vol. 1992 pp. 55-80. (AFOCEL, Paris).

Cauvin, B., Potts, B.M. and Potts, W.C. (1987). *Eucalyptus* : Hybridation artificielle - barrieres et hérédité des caracteres. *In* 'Annales de Recherches Sylvicoles'. Vol. 1986 pp. 255-303. (AFOCEL, Paris).

Chambers, P.G.S., Borralho, N.M.G. and Potts, B.M. (1996). Genetic analysis of survival in *Eucalyptus globulus* ssp. *globulus*. *Silvae Genetica* 45, 107-112.

Chambers, P.G.S., Potts, B.M. and Tilyard, P. (1997). The genetic control of flowering precocity in *Eucalyptus globulus* ssp. *globulus*. *Silvae Genetica* 46, 207-214.

Chaperon, H. (1977). Amélioration génétique des *Eucalyptus* hybrides au Congo Brazzaville. *In* 'Third World Consultation on Forest Tree Breeding'. *Proc. of* FAO Conf. Canberra, Australia, 21-26 Mar. 1977 pp. 1055-1070. (CSIRO Canberra).

Comer, C.W., Rockwood, D.L. and Riekerk, H. (1983). Frost tolerant *Eucalyptus* for north Florida. *In* 'Colloque international sur les *Eucalyptus* résistants au Froid'. *Proc. of* IUFRO/AFOCEL Conf. Bordeaux, France pp. 377-382. (AFOCEL, Paris).

Comstock, R.E., Robinson, H.F. and Harvey, P.H. (1949). A breeding procedure designed to make maximum use of both general and specific combining ability. *Agronomy Journal* 41, 360-367.

Cotterill, P.P. (1984). A plan for breeding radiata pine. *Silvae Genetica* 33, 84-90.

Cotterill, P.P. (1986a). Breeding Strategy: Don't underestimate simplicity! *In Proc. of* IUFRO Joint Meeting of Working Parties on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, USA, 12-17 Oct. 1986 pp. 8-23. (North Carolina State University, Raleigh).

Cotterill, P.P. (1986b). Genetic gains expected from alternative breeding strategies including simple low cost options. *Silvae Genetica* 35, 212-223.

Cotterill, P.P. and Brindbergs, M.L. (1997). Growth of first and second generation *Eucalyptus globulus* clonal cuttings and seedlings. *In* 'Silviculture and Improvement of Eucalypts'. *Proc. of* IUFRO/EMBRAPA Conf. Salvador, Bahia, Brazil, 24-29 Aug. 1997. (Eds. A.R. Higa, E. Schaitza and S. Gaiad) pp. 233-238. (EMBRAPA, Colombo).

Cotterill, P.P. and Brolin, A. (1997). Improving *Eucalyptus* wood, pulp and paper quality by genetic selection. *In* 'Silviculture and Improvement of Eucalypts'. *Proc. of* IUFRO/EMBRAPA Conf. Salvador, Bahia, Brazil, 24-29 Aug. 1997. (Eds. A.R. Higa, E. Schaitza and S. Gaiad) pp. 1-13. (EMBRAPA, Colombo).

Cotterill, P.P., Macrae, S. and Brolin, A. (1999). Growing eucalypt for high-quality papermaking fibres. *Appita* 52, 79-83.

Cremiere, L. (1988). Use of *Eucalyptus* hybrids in clonal plantation: Results 10 years after the first plantations in Congo. *In Proc. of* 10th Meeting, Research Working Group 1 (Forest Genetics) of the Australian Forestry Council. Gympie, Queensland, 30 Oct. - 4 Nov. 1988. (QFRI, Brisbane).

Cromer, R.N., Raupach, M., Clarke, A.R.P. and Cameron, J.N. (1975). Eucalypt plantations in Australia - the potential for intensive production and utilization. *Appita* 29, 165-173.

Dale, G.T., Aitken, K. and Sasse, J. (2000). Development of salt-tolerant *E. camaldulensis* × *E. grandis* hybrid clones using phenotypic selection and genetic mapping. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 227-233. (Queensland Department of Primary Industries, Brisbane).

Darrow, W.K. (1983). Provenance studies of frost resistant *Eucalyptus* in South Africa. *In* 'Colloque international sur les *Eucalyptus* résistants au Froid'. *Proc. of* IUFRO/AFOCEL Conf. Bordeaux, France pp. 448-468. (AFOCEL, Paris).

Darrow, W.K. (1995). A study of eucalypt clonal and hybrid material planted in the sub-humid zones of Kwazulu-Natal: Results of the first six years. *Institute for Commercial Forestry Research Bulletin Series* 2/95, 1-23.

Davidson, J. (1977). Advances from international cooperation on tropical *Eucalyptus*. *In* 'Third World Consultation on Forest Tree Breeding'. *Proc. of* FAO Conf. Canberra, Australia, 21-26 Mar. 1977 pp. 355-370. (CSIRO Canberra).

Davidson, N.J. and Reid, J.B. (1987). The influence of hardening and waterlogging on the frost resistance of subalpine eucalypts. *Australian Journal of Botany* 35, 91-101.

de Assis, T.F. (2000). Production and use of *Eucalyptus* hybrids for industrial purposes. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 63-74. (Queensland Department of Primary Industries, Brisbane).

de Little, D.W., Tibbits, W.N., Rasmussen, G.F. and Ravenwood, I.C. (1993). Genetic improvement strategy for APPM eucalypt tree farms in Tasmania. *In* 'Mass production technology for genetically improved fast growing forest tree species'. *Proc. of* AFOCEL/IUFRO Conference. Bordeaux. France, 14-18 Sep. 1992. (Eds. M. Noelle Bey, M. Leroy and S. Vérité) pp. 275-282. (AFOCEL, Paris).

Dean, G.H. (1995). Objectives for wood and fibre quality and uniformity. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 5-9. (CRC for Temperate Hardwood Forestry, Hobart).

Dean, G.H., French, J. and Tibbits, W.N. (1990). Variation in pulp and papermaking characteristics in a field trial of *Eucalyptus globulus*. *Proc. of* 44th Appita Annual General Conference. Rotorua, New Zealand,, 2-6 Apr. 1990 pp. 1-33. (APPITA, Melbourne).

Denison, N.P. and Kietzka, J.E. (1992). The use and importance of hybrid intensive forestry in South Africa. *In* 'Breeding Tropical trees. Resolving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species'. *Proc. of* IUFRO Conference S2.02.-08. Cartagena and Cali, Colombia, 9-18 October. (Eds. C.C. Lambeth and W. Dvorak) pp. 348-358. (IUFRO, Rome).

Dieters, M.J. and Dungey, H.S. (2000). Relationship between the relative importance of non-additive variance and the genetic correlation between hybrid and parental populations in some *Pinus* species. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 87-92. (Queensland Department of Primary Industries, Brisbane).

Dieters, M.J., White, T.L. and Hodge, G.R. (1995). Genetic parameter estimates for volume from full-sib tests of slash pine (*Pinus elliottii*). *Canadian Journal of Forest Research* 25, 1397-1408.

Dungey, H.S. (1991). 'Hybridisation between *Eucalyptus nitens* and *E. globulus*.' Unpublished B. Sc. (Hons.) Thesis, University of Tasmania.

Dungey, H.S. (2001). Pine hybrids - a review of their use performance and genetics. *Forest Ecology and Management* 148, 243-258.

Dungey, H.S., Dieters, M.J., Gwaze, D.P., Toon, P.G. and Nikles, D.G. (2000a). Interspecific pine hybrids. II. Genotype by environment interactions across Australia, Swaziland and Zimbabwe. *Forest Genetics* 7, 19-28.

Dungey, H.S., Dieters, M.J. and Nikles, D.G.E. (2000b). Hybrid Breeding and Genetics of Forest Trees. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000 p. 539. (Queensland Department of Primary Industries, Brisbane).

Dungey, H.S., Kerr, R.J. and Dieters, M.J. (2000c). A simulation of the comparative gains from three different hybrid tree breeding schemes. (II) Results. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 351-356. (Queensland Department of Primary Industries, Brisbane).

Dungey, H.S. and Nikles, D.G. (2000). An international survey of interspecific hybrids in forestry. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 419-425. (Queensland Department of Primary Industries, Brisbane).

Dungey, H.S. and Potts, B.M. (2002). Susceptibility of some *Eucalyptus* species and their hybrids to possum damage. *Australian Forestry* (in press).

Dungey, H.S., Potts, B.M., Carnegie, A.J. and Ades, P.K. (1997). *Mycosphaerella* leaf disease: Genetic variation in damage to *Eucalyptus nitens, E. globulus* and their F_1 hybrid. *Canadian Journal of Forest Research* 27, 750-759.

Dutkowski, G.W. (1995). Genetic variation in drought susceptibility of *Eucalyptus globulus* ssp. *globulus* plantations in Western Australia. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 199-203. (CRC for Temperate Hardwood Forestry, Hobart).

Dutkowski, G.W. and Potts, B.M. (1999). Geographical patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. *Australian Journal of Botany* 46, 237-263.

Dutkowski, G.W., Potts, B.M. and Borralho, N.M.G. (1997). Revised racial classification of *Eucalyptus globulus* ssp. *globulus* and the importance of including race in analysis of progeny trials. *In* 'Silviculture and Improvement of Eucalypts'. *Proc. of* IUFRO/EMBRAPA Conf. Salvador, Bahia, Brazil, 24-29 Aug. 1997. (Eds. A.R. Higa, E. Schaitza and S. Gaiad) pp. 322-329. (EMBRAPA, Colombo).

Dutkowski, G.W., Potts, B.M., Williams, D.R., Kube, P.D. and McArthur, C. (2001). Geographic genetic variation in Central Victorian *Eucalyptus nitens*. *In* 'Developing the Eucalypt of the Future'. *Proc. of* IUFRO International Symposium. Valdivia, Chile, 10-15 Sep. 2001. (Eds. S. Barros and R. Ipinza) . (INFOR, Valdivia; CD-ROM).

Dvorak, W.S., Franklin, E.C. and Meskimen, G. (1981). Breeding strategy for *E. robusta* in southern Florida. *In Proc. of* 16th Southern Forest Tree Improvement Conference. Blacksburg, Virginia, USA, 27-28 May 1981 pp. 116-122. (US Forest Service, Athens, Georgia).

Egerton-Warburton, L.M., Griffin, B.J. and Lamont, B.B. (1993). Pollen pistil interactions in *Eucalyptus calophylla* provide no evidence of a selection mechanism for aluminum tolerance. *Australian Journal of Botany* 41, 541-552.

Eldridge, K., Davidson, J., Harwood, C. and van Wyk, G. (1993). 'Eucalypt Domestication and Breeding'. (Clarendon Press, Oxford).

Eldridge, K.G. (1968). Physiological studies of altitudinal variation in *Eucalyptus regnans*. *Proceedings of the Ecological Society of Australia* 3, 70-76.

Eldridge, K.G. (1971). Genetically improved eucalypt seed for Australian pulpwood forests. *Appita* 25, 105-109.

Eldridge, K.G. (1972). 'Genetic variation in growth of *Eucalyptus regnans*.' Bulletin No. 46. (Forestry and Timber Bureau, Canberra).

Eldridge, K.G. (1995). Eucalypt base populations for selection. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 204-207. (CRC for Temperate Hardwood Forestry, Hobart).

Ellis, M.F. (1991). 'Breeding Systems and Interspecific Hybridisation in the Genus *Eucalyptus* L'Her.' Unpublished PhD Thesis, University of South Australia.

Ellis, M.F., Sedgley, M. and Gardner, J.A. (1991). Interspecific pollen-pistil interaction in *Eucalyptus* L'Her. (Myrtaceae): The effect of taxonomic distance. *Annals of Botany* 68, 185-194.

Endo, M. and Lambeth, C.C. (1992). Promising potential of the hybrid *Eucalyptus grandis* x *E. urophylla* over *E. grandis* in Colombia. *In* 'Breeding Tropical trees. Resolving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species'. *Proc. of* IUFRO Conference S2.02.-08. Cartagena and Cali, Colombia, 9-18 October. (Eds. C.C. Lambeth and W. Dvorak) pp. 366-371. (IUFRO, Rome).

England, N.F. and Borralho, N.M.G. (1995). Heritability of rooting success in *Eucalyptus globulus* stem cuttings. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 237-238. (CRC for Temperate Hardwood Forestry, Hobart).

Espejo, J.E., England, N.F. and Griffin, A.R. (1995). Results of a crossing program with *Eucalyptus nitens* and *E. globulus* in Chile. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 239-240. (CRC for Temperate Hardwood Forestry, Hobart).

Evans, J. (1983). Choice of *Eucalyptus* species and provenances in cold temperate atlantic climates. *In* 'Colloque international sur les *Eucalyptus* résistants au Froid'. *Proc. of* IUFRO/AFOCEL Conf. Bordeaux, France pp. 255-274. (AFOCEL, Paris).

Falconer, D.S. and MacKay, T.F.C. (1996). 'Introduction to Quantitative Genetics'. 4th Ed. (Longman Scientific and Technical, New York).

Fonseca, S.M., Oliveira, R.C. and Silveira, P.N. (1995). Industrial tree selection: procedures, risks, costs and benefits. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 14-19. (CRC for Temperate Hardwood Forestry, Hobart).

Franclet, A. and Boulay, M. (1982). Micropropagation of frost resistant eucalypt clones. *Australian Forest Research* 13, 83-89.

Franklin, E.C. (1986). Estimation of genetic parameters through four generations of selection in *Eucalyptus grandis*. *In Proc. of* IUFRO Joint Meeting of Working Parties on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, USA, 12-17 Oct. 1986. pp. 200-209. (North Carolina State University, Raleigh).

Gallais, A. (1988a). Heterosis: its genetic basis and its utilisation in plant breeding. *Euphytica* 39, 95-104.

Gallais, A. (1988b). Prediction of mean and variance of hybrids and of lines that can be derived from a random mating population. *Theoretical and Applied Genetics* 75, 625-627.

Gardiner, C.A. and Crawford, D.A. (1987). '1987 Seed Collections of *Eucalyptus globulus* subsp. *globulus* for Tree Improvement Purposes'. (CSIRO Division of Forest Research, Canberra).

Gardiner, C.A. and Crawford, D.A. (1988). '1988 Seed Collections of *Eucalyptus globulus* subsp. *globulus* for Tree Improvement Purposes'. (CSIRO Division of Forest Research, Canberra).

Gea, L.D., McConnochie, R. and Borralho, N.M.G. (1997). Genetic parameters for growth and wood density traits in *Eucalyptus nitens* in New Zealand. *New Zealand Journal of Forestry Science* 27, 237-244.

Gilmour, A.R., Cullis, B.R., Welham, S.J. and Thompson, R. (1999). 'ASREML.' Technical Report No. (NSW Agriculture, Orange).

Goodnight, C.J. (1988). Epistasis and the effect of founder events on the additive genetic variance. *Evolution* 42, 441-454.

Gordon, I.L. (1999). Quantitative genetics of intraspecies hybrids. *Heredity* 83, 757-764.

Gore, P.L. and Potts, B.M. (1995). The genetic control of flowering time in *Eucalyptus globulus, E. nitens* and their F1 hybrids. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 241-242. (CRC for Temperate Hardwood Forestry, Hobart).

Gore, P.L., Potts, B.M., Volker, P.W. and Megalos, J. (1990). Unilateral cross-incompatibility in *Eucalyptus* : the case of hybridisation between *E. globulus* and *E. nitens*. *Australian Journal of Botany* 38, 383-394.

Greaves, B.L. (1994). 'Co-operative eucalypt breeding within the Southern Tree Breeding Association: how to share existing genetic resources.' Technical Report No. 94/15. (APM Forests, Morwell, Victoria).

Greaves, B.L. and Borralho, N.M.G. (1996). The influence of basic density and pulp yield on the cost of eucalypt kraft pulping: a theoretical model of tree breeding. *Appita* 49, 90-95.

Greaves, B.L., Borralho, N.M.G. and Raymond, C.A. (1995). Use of a Pilodyn for indirect selection of basic density in *Eucalyptus nitens*. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 106-109. (CRC for Temperate Hardwood Forestry, Hobart).

Greaves, B.L., Borralho, N.M.G. and Raymond, C.A. (1997a). Breeding objectives for plantation eucalypts grown for production of kraft pulp. *Forest Science* 43, 465-472.

Greaves, B.L., Borralho, N.M.G., Raymond, C.A., Evans, R. and Whiteman, P. (1997b). Age-age correlations in, and relationships between basic density and growth in *Eucalyptus nitens*. *Silvae Genetica* 46, 264-270.

Greaves, B.L., Borralho, N.M.G., Raymond, C.A. and Farrington, A. (1996). Use of a pilodyn for the indirect selection of basic density in *Eucalyptus nitens*. *Canadian Journal of Forest Research* 26, 1643-1650.

Griffin, A.R. (1990). Effects of inbreeding on growth of forest trees and implication for management of seed supplies for plantation programmes. *In* 'Reproductive Ecology of Tropical Forest Plants'. (Eds. K.S. Bawa and M. Hadley) pp. 355-372. (UNESCO and The Parthenon Publishing Group, Paris, France).

Griffin, A.R., Burgess, I.P. and Wolf, L. (1988). Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'Herit. - a review. *Australian Journal of Botany* 36, 41-66.

Griffin, A.R., Ching, K.K., Johnson, K.W., Hand, F.C. and Burgess, I.P. (1982a). Processing *Eucalyptus* pollen for use in controlled pollination. *Silvae Genetica* 31, 198-203.

Griffin, A.R. and Cotterill, P.P. (1988). Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucalyptus regnans* and some implications for breeding strategy. *Silvae Genetica* 37, 124-131.

Griffin, A.R., Harbard, J.L., Centurion, C. and Santini, P. (2000). Breeding *Eucalyptus grandis* × *globulus* and other inter-specific hybrids with high inviability - problem analysis and experience with Shell Forestry projects in Uruguay and Chile. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 1-13. (Queensland Department of Primary Industries, Brisbane).

Griffin, A.R., Whitemen, P., Rudge, T., Burgess, I.P. and Moncur, M.W. (1993). Effect of paclobutrazol on flower-bud production and vegetative growth in two species of *Eucalyptus*. *Canadian Journal of Forest Research* 23, 630-647.

Griffin, A.R., Williams, E.R. and Johnson, K.W. (1982b). Early height growth and frost hardiness of *Eucalyptus regnans* provenances in twelve field trials in south-east Australia. *Australian Forest Research* 12, 263-79.

Gwaze, D.P., Dungey, H.S., Dieters, M.J., Toon, P.G. and Nikles, D.G. (2000). Interspecific pine hybrids. I. Genetic parameter estimates in Australia. *Forest Genetics* 7, 11-20.

Haines, R.J. (2000). Clonal forestry in Queensland and implications for hybrid breeding strategies. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 386-389. (Queensland Department of Primary Industries, Brisbane).

Hallam, P.M. and Reid, J.B. (1989). Seasonal and genetic variation in frost hardiness of *Eucalyptus delegatensis*. *Canadian Journal of Forest Research* 19, 480-488.

Hallam, P.M. and Tibbits, W.N. (1988). Determination of frost hardiness of *Eucalyptus* using the electrical conductivity of diffusate in conjunction with a freezing chamber. *Canadian Journal of Forest Research* 18, 595-600.

Harbard, J.L., Griffin, A.R. and Espejo, J. (1999). Mass controlled pollination of *Eucalyptus globulus*: a practical reality. *Canadian Journal of Forest Research* 29, 1457-1463.

Hardner, C.M. (1996). 'Inbreeding in Three Forest Eucalypts.' Unpublished PhD Thesis, University of Tasmania.

Hardner, C.M. and Potts, B.M. (1995a). Inbreeding depression and changes in variation after selfing *Eucalyptus globulus* subsp. *globulus*. *Silvae Genetica* 44, 46-54.

Hardner, C.M. and Potts, B.M. (1995b). The influence of mortality and stand age on the estimation of genetic parameters in *Eucalyptus regnans*. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 217-221. (CRC for Temperate Hardwood Forestry, Hobart).

Hardner, C.M. and Potts, B.M. (1997). Post-dispersal selection under mixed-mating in *Eucalyptus regnans*. *Evolution* 51, 103-111.

Hardner, C.M., Potts, B.M. and Gore, P.L. (1995). Relationship between cross success and the geographical proximity of *Eucalyptus globulus* ssp. globulus parents. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 243-244. (CRC for Temperate Hardwood Forestry, Hobart).

Hardner, C.M., Potts, B.M. and Gore, P.L. (1998). The relationship between cross success and spatial proximity of *Eucalyptus globulus* ssp. *globulus* parents. *Evolution* 52, 614-618.

Hardner, C.M. and Tibbits, W.N. (1998). Inbreeding depression for growth and fecundity traits in *Eucalyptus nitens*. *Forest Genetics* 5, 11-20.

Hardner, C.M., Vaillancourt, R.E. and Potts, B.M. (1996). Stand density influences outcrossing rate and growth of open-pollinated families of *Eucalyptus globulus*. *Silvae Genetica* 45, 226-228.

Harfouche, A., Bahrman, N., Baradat, P., Guyon, J.P., Petit, R.J. and Kremer, A. (2000). Provenance hybridization in a diallel mating scheme of maritime pine (*Pinus pinaster*). II. Heterosis. *Canadian Journal of Forest Research* 30, 10-16.

Harfouche, A. and Kremer, A. (2000). Provenance hybridization in a diallel mating scheme of maritime pine (*Pinus pinaster*). I. Means and variance components. *Canadian Journal of Forest Research* 30, 1-9.

Harwood, C.E. (1980). Frost resistance of subalpine *Eucalyptus* species I. Experiments using a radiation frost room. *Australian Journal of Botany* 28, 587-599.

Henderson, C.R. (1975). Best linear unbiased estimation and prediction under a selection model. *Biometrics* 32, 69.

Hetherington, S. and Orme, R.K. (1990). Seedling variation in root formation of *Eucalyptus globulus* cuttings. *Proceedings of the International Plant Propagators' Society* 39, 56-60.

Higa, A.R. (1986). Frost resistance genetic variation between and within *E. viminalis* populations. *In Proc. of* 9th Meeting of Research Working Group 1 (Forest Genetics) of the Australian Forestry Council. Somerset, Tasmania. (Eds. A.R. Griffin and P.W. Volker). p. 106. (CSIRO, Canberra).

Higa, A.R., Schaitza, E. and Gaiad, S. (Eds.) (1997). 'Silviculture and Improvement of Eucalypts.' *In Proc. of* IUFRO/EMBRAPA Conf. Salvador, Bahia, Brazil, 24-29 Aug. 1997. (EMBRAPA, Colombo).

Hillis, W.E. and Brown, A.G. (Eds.) (1984). 'Eucalypts for Wood Production.' . (Academic Press, Melbourne, Sydney).

Hodge, G.R., Volker, P.W., Potts, B.M. and Owen, J.V. (1996). A comparison of genetic information from open-pollinated and control-pollinated progeny tests in two eucalypt species. *Theoretical and Applied Genetics* 92, 53-63.

Hyun, S.K. (1976). Interspecific hybridization in pines with the special reference to *Pinus rigida x taeda*. *Silvae Genetica* 25, 188-191.

Ipinza, R.H., García, X., Apiolaza, L.A., Molina, M.P., Chung, P. and Parra, P. (1994). Variación juvenil de un ensayo de procedencias y familias de *Eucalyptus globulus* subsp. *globulus* Labill, en la séptima región, Chile. *Ecología* 8, 259-270.

Jarvis, S.F., Borralho, N.M.G. and Potts, B.M. (1995). Implementation of a multivariate BLUP model for genetic evaluation of *Eucalyptus globulus* in Australia. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 212-216. (CRC for Temperate Hardwood Forestry, Hobart).

Jayawickrama, K.J.S., Schlatter V, J.E. and Escobar R, R. (1993). Eucalypt plantation forestry in Chile. *Australian Forestry* 56, 179-192.

Johnston, J.A., Grise, D.J., Donovan, L.A. and Arnold, M.L. (2001). Environment-dependent performance and fitness of *Iris brevicaulis, I. fulva* (*Iridaceae*) and hybrids. *American Journal of Botany* 88, 933-938.

Jordan, G., Potts, B.M. and Wiltshire, R. (1999). Strong, independent quantitative genetic control of vegetative phase change and first flowering in *Eucalyptus globulus* ssp. *globulus*. *Heredity* 83, 179-187.

Jordan, G.J., Borralho, N.M.G., Tilyard, P. and Potts, B.M. (1994). Identification of races in *Eucalyptus globulus* spp *globulus* based on growth traits in Tasmania and geographic distribution. *Silvae Genetica* 43, 292-298.

Jordan, G.J., Potts, B.M., Chalmers, P. and Wiltshire, R.J.E. (2000). Quantitative genetic evidence that the timing of vegetative phase change in *Eucalyptus globulus* ssp. *globulus* is an adaptive trait. *Australian Journal of Botany* 48, 561-567.

Jordan, G.J., Potts, B.M., Kirkpatrick, J.B. and Gardiner, C. (1993). Variation in the *Eucalyptus globulus* complex revisited. *Australian Journal of Botany* 41, 763-85.

Kageyama, P.Y. and Kikuti, P. (1989). Comparison between clones and open-pollinated progenies originating from a population of *Eucalyptus grandis* (Hill) Maiden in Brazil. *In* 'Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry'. *Proc. of* IUFRO Conf. S2.02. -08. Pattaya, Thailand, Nov. 1988. (Eds. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 227-235. (Oxford Forestry Institute, Oxford, United Kingdom; Winrock Interantional, Arlington, Virginia, USA).

Kedharnath, S. and Vakshasya, R.K. (1978). Estimate of components of variance, heritability and correlation among some growth parameters in *Eucalyptus tereticornis*. *In* 'Third World Consultation on Forest Tree Breeding'. *Proc. of* FAO Conf. Canberra, Australia, 21-26 Mar. 1977. pp. 667-676. (CSIRO Canberra).

Kerr, R.J., Dungey, H.S. and Dieters, M.J. (2000). A simulation of the comparative gains from three different hybrid tree breeding schemes. I. Theory. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 343-350. (Queensland Department of Primary Industries, Brisbane).

Kibblewhite, R.P., Riddell, M.J.C. and Shelbourne, C.J.A. (1998). Kraft fibre and pulp qualities of 29 trees of New Zealand grown *Eucalyptus nitens*. *Appita* 51, 114-121.

King, J.N., Carson, M.J. and Johnson, G.R. (1998). Analysis of disconnected diallel mating designs. Results from a third generation progeny test of the New Zealand radiata pine improvement programme. *Silvae Genetica* 47, 80-87.

Kinghorn, B. (1980). The expression of "recombination loss" in quantitative traits. *Zeitschrift fuer Tierzuechtung und Zuechtungsbiologie* 97, 138-143.

Klekowski, E.J. (1988). Genetic load and its causes in long-lived plants. *Trees* 2, 195-203.

Kremer, A. (1981). Déterminisme génétique de la croissance en hauter du pin maritime. III. Evolution des composantes de la variance phénotypique et génotypique. *Annales de Sciencias Forestiere* 38, 355-375.

Kube, P.D., Borralho, N.M.G., Bail, I., Tibbits, W.N. and McConnochie, R. (1996). 'Genetic parameters of *Eucalyptus nitens* for growth in Australia and New Zealand.' Report No. 18. (NZ FRI / Industry Eucalypt Breeding Co-operative, Rotorua).

Kube, P.D. and Raymond, C.A. (2002). Selection strategies for genetic improvement of basic density in *Eucalyptus nitens*. *Forest Genetics* (in press).

Kube, P.D., Raymond, C.A. and Banham, P.W. (2000). 'Genetic parameters and predicted gains for *Eucalyptus nitens* wood fibre production in Tasmania'. CRCSPF Technical Report. Vol. 43. (CRC Sustainable Production Forestry, Hobart, Tasmania).

Ledig, F.T. (1986). Heterozygosity, heterosis, and fitness in outbreeding plants. *In* 'Conservation Biology. The Science of Scarcity and Diversity'. (Ed. M.E. Soulé) pp. 77-104. (Sinauer Associates, Sunderland, Massachusetts).

Levin, D.A. (1978). Hybridization: An Evolutionary Perspective. *In* 'Benchmark Papers in Genetics'. Vol. 11. (Ed. D.L. Jameson). pp. 311. (Dowden, Hutchinson & Ross Inc., Pennsylvania).

Liu, X., Yang, M., Wang, G., Pegg, R.E. and Arnold, R.J. (1996). Development and genetic improvement of China's eucalypt resource. *In* 'Tree Improvement for Sustainable Tropical Forestry'. *Proc. of* QFRI-IUFRO Conf. Caloundra,, Queensland, Australia., 27 Oct. - 1 Nov. 1996. (Eds. M.J. Dieters, A.C. Matheson, D.G. Nikles, C.E. Harwood and S.M. Walker). pp. 92-93. (QFRI, Brisbane).

Lo, L.L., Fernando, R.L. and Grossman, M. (1993). Covariance between relatives in multibreed populations: additive model. *Theoretical and Applied Genetics* 87, 423-430.

Lopez, G., Potts, B.M., Dutkowski, G.W., Apiolaza , L.A. and Gelid, P. (2001a). Genetic variation and inter-trait correlations in *Eucalyptus globulus* base population trials in Argentina. *Forest Genetics* (submitted).

Lopez, G.A., Apiolaza, L.A., Potts, B.M., Dutkowski, G.W., Gelid, P. and Rodriguez Traverso, J.M. (2001b). Genetic parameters for growth and pilodyn from *Eucalyptus globulus* in Argentina. *In* 'Developing the Eucalypt of the Future'. *Proc. of* IUFRO International Symposium. Valdivia, Chile, 10 - 15 Sep. 2001. (Eds. S. Barros and R. Ipinza). (INFOR, Valdivia; CD-ROM).

Lopez, G.A., Potts, B.M. and Tilyard, P.A. (2000). The performance of F_1 hybrids between *E. ovata* and *E. globulus* ssp. *globulus* compared to inbred and outcrossed controls. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles). pp. 457-461. (Queensland Department of Primary Industries, Brisbane).

Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* 45, 622-629.

MacDonald, A.C., Borralho, N.M.G. and Potts, B.M. (1997). Genetic variation for growth and wood density in *Eucalyptus globulus* ssp. *globulus* in Tasmania (Australia). *Silvae Genetica* 46, 236-241.

MacDonald, A.C., Borralho, N.M.G., Potts, B.M. and Jarvis, S.F. (1995). International analysis of a comprehensive *Eucalyptus globulus* Labill. ssp. *globulus* base population collection. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 247-248. (CRC for Temperate Hardwood Forestry, Hobart).

MacRae, S. and Cotterill, P.P. (1997). Macropropagation and micropropagation of *Eucalyptus globulus*: Means of capturing genetic gain. *In* 'Silviculture and Improvement of Eucalypts'. *Proc. of* IUFRO/EMBRAPA Conf. Salvador, Bahia, Brazil, 24-29 Aug. 1997. (Eds. A.R. Higa, E. Schaitza and S. Gaiad) pp. 102-110. (EMBRAPA, Colombo).

Malan, F.S. (1988). Genetic variation in some growth and wood properties among 18 full-sib families of South African grown *Eucalyptus grandis*: A preliminary investigation. *South African Forestry Journal* 146, 38-43.

Marcó, M.A., Pujato, J. and Marlats, R.M. (1991). Frost tolerance in a seven-month-old provenance trial of *Eucalyptus grandis* (Hill) Maiden in Argentina. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schönau) pp. 426-432. (Southern African Institute of Forestry, Pretoria).

Martin, B. (1989). The benefits of hybridization. How do you breed for them? *In* 'Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry'. *Proc. of* IUFRO Conf. S2.02. -08. Pattaya, Thailand, Nov. 1988. (Eds. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 79-92. (Oxford Forestry Institute, Oxford, United Kingdom; Winrock Interantional, Arlington, Virginia, USA).

Matheson, A.C. (1990). Breeding strategies for MPTs. *In* 'Tree Improvement of Multipurpose Species'. Multipurpose Tree Species Network Technical Series. Vol. 2. (Eds. G. Nancy and N. Adams). pp. 67-99. (Winrock International Institute for Agricultural Development, Arlington, Virginia).

Mayo, O. (1987). 'The Theory of Plant Breeding'. 2nd Ed. (Clarendon Press, Oxford).

McComb, J.A., Oddie, R.L.A., Chapman, S.R., Calver, M.C., Rasmussen, G.F., Hingston, T.L. and de Little, D.W. (2000). Salt tolerant hybrid eucalypts for wood fibre production. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 252-258. (Queensland Department of Primary Industries, Brisbane).

Meskimen, G.F., Rockwood, D.L. and Reddy, K.V. (1987). Development of *Eucalyptus* clones for a summer rainfall environment with periodic severe frosts. *New Forests* 3, 197-205.

Meyer, K. (1989). Restricted maximum likelihood to estimate variance components for animal models with several random effects using a derivative-free algorithm. *Genetic Selection and Evolution* 21, 317-340.

Muneri, A. and Raymond, C.A. (2001). Genetic parameters and genotype-by-environment interactions for basic density, pilodyn penetration and diameter in *Eucalyptus globulus*. *Forest Genetics* 7, 321-332.

Myburg, A., Griffin, A.R., Sederoff, R. and Whetten, R. (2000). Genetic analysis of interspecific backcross families of a hybrid of *Eucalyptus grandis* and *Eucalyptus globulus*. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 462-466. (Queensland Department of Primary Industries, Brisbane).

Namkoong, G. (1979). 'Introduction to Quantitative Genetics in Forestry'. U.S. Dept. of Agriculture Technical Bulletin No.1588. (U.S. Govt. Printing Office, Washington, D.C.).

Nelder, J.A. and Mead, R. (1965). A simple method for function minimization. *Computer Journal* 7, 147-151.

Newman, S. and Reverter, A. (2000). Practical aspects of crossbred genetic evaluation. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 303-315. (Queensland Department of Primary Industries, Brisbane).

Nicholas, F.W. (1987). 'Veterinary Genetics'. (Clarendon Press, Oxford).

Nicholls, J.W.P. and Pederick, L.A. (1979). Variation in some wood characteristics of *Eucalyptus nitens*. *Australian Forest Research* 9, 309-321.

Nikles, D.G. (1992). Hybrids of forest trees: The basis of hybrid superiority and a discussion of breeding methods. *In* 'Resolving Tropical Forest Resource Concerns Through Tree Improvement, Gene Conservation and Domestication of New Species'. *Proc. of* IUFRO Conf. S2.02.-08 Breeding Tropical Trees. Cartagena and Cali, Colombia, 9-18 October. (Eds. C.C. Lambeth and W. Dvorak) pp. 333-347. (IUFRO, Rome).

Nikles, D.G. (2000). Experience with some *Pinus* hybrids in Queensland, Australia. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 27-43. (Queensland Department of Primary Industries, Brisbane).

Nikles, D.G. and Griffin, A.R. (1992). Breeding hybrids of forest trees: definitions, theory, some practical examples, and guidelines on strategy with tropical acacias. *ACIAR Proceedings* 37, 101-109.

Nikles, D.G. and Newton, R.S. (1991). Correlations of breeding values in pure and hybrid populations of hoop pine and some southern pines in Queensland and relevance to breeding strategies. *In Proc. of* 11th Meeting of the Research Working Group 1 (Forest Genetics) of the Australian Forestry Council. Coonawarra, South Australia, Mar. 1991. (Ed. C.A. Dean) pp. 1-5. (CSIRO, Mt. Gambier).

Nikles, D.G. and Robinson, M.J. (1989). The development of *Pinus* hybrids for operational use in Queensland. *In* 'Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry'. *Proc. of* IUFRO Conf. S2.02. -08. Pattaya, Thailand, Nov. 1988. (Eds. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 272-282. (Oxford Forestry Institute, Oxford, United Kingdom; Winrock Interantional, Arlington, Virginia, USA).

Orme, R.K. (1977). *Eucalyptus globulus* provenances. *In* 'Third World Consultation on Forest Tree Breeding'. *Proc. of* FAO Conf. Canberra, Australia, 21-26 Mar. 1977. pp. 207-222. (CSIRO Canberra).

Orr, H.A. (1995). The population genetics of speciation: The evolution of hybrid incompatibilities. *Genetics* 139, 1805-1813.

Paramathma, M., Surendran, C. and Vinaya Rai, R.S. (1997). Studies of heterosis in six *Eucalyptus* species. *Journal of Tropical Forest Science* 9, 283-293.

Paterson, H.D. and Williams, E.R. (1976). A new class of resolvable incomplete block designs. *Biometrika* 63, 83-92.

Paton, D.M. (1981). *Eucalyptus* physiology. III. Frost resistance. *Australian Journal of Botany* 29, 675-688.

Patterson, H.D. and Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika* 58, 545-554.

Pederick, L.A. (1979). Natural variation in Shining Gum (*Eucalyptus nitens*). *Australian Forest Research* 9, 41-63.

Perrow (MacRae), S. and Cotterill, P. (2000). Breeding strategies for the next generation of clonal forests. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 44-47. (Queensland Department of Primary Industries, Brisbane).

Pilipenka, F.S. (1969). 'Hybridisation of eucalypts in the USSR'. Akademiya Nauk SSSR. Botanicheski Institut. Trudy 6th series. Introduktsiya Rastenii Zelende Stroitel'stvd. Vol. 9.

Potts, B.M. (1990). Short note: A rare dwarf mutant of *Eucalyptus gunnii*. *Silvae Genetica* 39, 84-86.

Potts, B.M., Borralho, N.M.G., Reid, J.B., Cromer, R.N., Tibbits, W.N. and Raymond, C.A. (Eds.) (1995a). 'Eucalypt Plantations, Improving Fibre Yield and Quality.' *Proc. of* CRCTHF-IUFRO Conference. Hobart, Tasmania, Australia. 19-24 Feb. 1995. (CRC for Temperate Hardwood Forestry, Hobart).

Potts, B.M. and Dungey, H.S. (2001). Hybridisation of *Eucalyptus*: Key issues for breeders and geneticists. *In* 'Developing the Eucalypt of the Future'. *Proc. of* IUFRO International Symposium. Valdivia, Chile, 10 - 15 Sep. 2001. (Eds. S. Barros and R. Ipinza) . (INFOR, Valdivia; CD-ROM).

Potts, B.M. and Jordan, G.J. (1994a). Genetic variation in the juvenile leaf morphology of *Eucalyptus globulus* Labill. ssp. *globulus*. *Forest Genetics* 1, 81-95.

Potts, B.M. and Jordan, G.J. (1994b). Spatial pattern and scale of variation in *Eucalyptus globulus* Labill. ssp. *globulus*: Variation in seedling abnormalities and early growth. *Australian Journal of Botany* 42, 471-492.

Potts, B.M. and Marsden-Smedley, J.B. (1989). *In vitro* germination of *Eucalyptus* pollen: response to variation in boric acid and sucrose. *Australian Journal of Botany* 37, 429-441.

Potts, B.M. and Potts, W.C. (1986). Eucalypt breeding in France. *Australian Forestry* 49, 210-218.

Potts, B.M., Potts, W.C. and Cauvin, B. (1987). Inbreeding and interspecific hybridisation in *Eucalyptus gunnii*. *Silvae Genetica* 36, 194-198.

Potts, B.M., Potts, W.C. and Cauvin., B. (1986). Artificial and natural hybridization of *Eucalyptus gunnii*. *In Proc. of* 9th Meeting of Research Working Group 1 (Forest Genetics) of the Australian Forestry Council. Burnie, Tasmania. (Eds. A.R. Griffin and P.W. Volker) . (CSIRO, Canberra).

Potts, B.M., Volker, P.W. and Dungey, H.S. (1992). Barriers to the production of interspecific hybrids in *Eucalyptus*. *In* 'Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species'. *Proc. of* AFOCEL-IUFRO Symposium. Bordeaux, France. pp. 193-204. (AFOCEL, Paris).

Potts, B.M., Volker, P.W., Hodge, G.R., Borralho, N.M.G., Hardner, C.H. and Owen, J.V. (1995b). Genetic limitations to the exploitation of base populations of *Eucalyptus globulus* ssp. *globulus*. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 217-221. (CRC for Temperate Hardwood Forestry, Hobart).

Potts, B.M., Volker, P.W., Tilyard, P.A. and Joyce, K. (2000). The genetics of hybridisation in the temperate *Eucalyptus*. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 200-211. (Queensland Department of Primary Industries, Brisbane).

Potts, B.M. and Wiltshire, R.J.E. (1997). Eucalypt genetics and genecology. *In* 'Eucalypt Ecology: Individuals to Ecosystems'. (Eds. J. Williams and J. Woinarski) pp. 56-91. (Cambridge University Press, Cambridge).

Powell, M.B. (1993). Estimates of correlations between GCA's and GHA's in *Pinus* population hybrids. *Proc. of* 12th Meeting, Research Working Group 1 (Forest Genetics) of the Australian Forestry Council. Australia National University, Canberra, 15 -18 Feb. 1993. (Ed. M.U. Slee) pp. 101-103. (ANU Forestry Department, Canberra).

Powell, M.B. and Nikles, D.G. (1996). Genetic parameter estimates and predicted breeeding values for diameter, height and stem straightness of *Pinus elliottii, Pinus caribaea* var. *hondurensis* and their F_1 hybrid. *In* 'Tree Improvement for Sustainable Tropical Forestry'. *Proc. of* QFRI - IUFRO Breeding Tropical Trees Conf. Caloundra, Queensland, 27 Oct.-1 Nov. 1996. (Eds. M.J. Dieters, A.C. Matheson, D.G. Nikles, C.E. Harwood and S.M. Walker) pp. 169-172. (Queensland Forest Research Institute, Brisbane).

Pryor, L.D. (1961). Inheritance, selection, and breeding in eucalypts. *Proc. of* Second World *Eucalyptus* Conference. Sao Paulo, Brazil pp. 1-7. (FAO, Rome).

Pryor, L.D. (1978). Reproductive habits of the eucalypts. *Unasylva* 30, 42-46.

Pryor, L.D. and Boden, R.W. (1962). Blowflies as pollinators in producing *Eucalyptus* seed. *Australian Journal of Science* 24, 326.

Pryor, L.D. and Johnson, L.A.S. (1971). 'A Classification of the Eucalypts'. (Australian National University Press, Canberra).

Purnell, R.C. (1986). Early results from provenance / progeny trials of *Eucalyptus nitens* in South Africa. *In Proc. of* IUFRO Joint Meeting of Working Parties on Breeding Theory, Progeny Testing and Seed Orchards. Williamsburg, Virginia, USA., 12-17 Oct. 1986 pp. 500-513. (North Carolina State University, Raleigh).

Rasmussen, G.F., Bower, D.L.M. and Borralho, N.M.G. (1995). Genetic control of micro-propagation characteristics in *Eucalyptus nitens x E. globulus* and implications for selection of clones. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 289-292. (CRC for Temperate Hardwood Forestry, Hobart).

Raymond, C.A. (1995). Genetic control of wood and fibre traits in eucalypts. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 49-52. (CRC for Temperate Hardwood Forestry, Hobart).

Raymond, C.A., Harwood, C.E. and Owen, J.V. (1986). A conductivity method for screening populations of eucalypts for frost damage and frost tolerance. *Australian Journal of Botany* 34, 377-393.

Raymond, C.A. and MacDonald, A.C. (1998). Where to shoot your pilodyn: with tree variation in basic density in plantation *Eucalyptus globulus* and *E. nitens* in Tasmania. *New Forests* 15, 205-221.

Raymond, C.A. and Muneri, A. (2001). Non-destructive sampling of *Eucalyptus globulus* and *E. nitens* for wood properties. I. Basic density. *Wood Science and Technology* 35, 27-39.

Raymond, C.A., Muneri, A. and MacDonald, A.C. (1998). Non-destructive sampling for basic density in *Eucalyptus globulus* and *E. nitens*. *Appita* 51, 224-228.

Raymond, C.A., Owen, J.V., Eldridge, K.G. and Hardwood, C.E. (1992a). Screening eucalypts for frost tolerance in breeding programs. *Canadian Journal of Forest Research* 22, 1271-1277.

Raymond, C.A., Owen, J.V. and Ravenwood, I.C. (1992b). Genetic variation for frost tolerance in a breeding population of *Eucalyptus nitens*. *Silvae Genetica* 41, 355-362.

Resende, M.D.V., Vencovsky, R. and Fernandes, J.S.C. (1995). Selection and genetic gains in populations of *Eucalyptus* with a mixed mating system. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 191-193. (CRC for Temperate Hardwood Forestry, Hobart).

Rezende, G.D.S.P. and de Resende, M.D.V. (2000). Dominance effects in *Eucalyptus grandis, Eucalyptus urophylla* and hybrids. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Ed. H.S.D. Dungey, M.J. and Nikles, D.G.) pp. 93-100. (Queensland Department of Primary Industries, Brisbane).

Rockwood, D.L. (1991). Freeze resilient *E. grandis* clones for Florida, USA. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schonau) pp. 455-466. (Southern African Institute of Forestry, Pretoria).

Rojas Vergara, P., Ramirez de Arellano, P., Contardo, C., Balocchi, C. and Potts, B.M. (2001). Desarrollo de una metodologia para la produccion de semilla hibrida intra e interespecifica en un huerto semillero clonal de *Eucalyptus globulus*. *In* 'Developing the Eucalypt of the Future'. *Proc. of* IUFRO International Symposium. Valdivia, Chile, 10 - 15 Sep. 2001. (Eds. S. Barros and R. Ipinza) . (INFOR, Valdivia; CD-ROM).

Rook, D.A., Wilcox, M.D., Holden, D.G. and Warrington, I.J. (1980). Provenance variation in frost tolerance of *Eucalyptus regnans* F. Muell. *Australian Forest Research* 10, 213-238.

SASS (1987). 'ALPHAGEN. A program for constructing alpha-lattice incomplete block designs'. (Scottish Agricultural Statistical Service, Edinburgh).

Schmidtling, R.C. and Nelson, C.D. (1996). Interprovenance crosses in Loblolly pine using selected parents. *Forest Genetics* 3, 53-66.

Schneck, V. and Langner, W. (2000). A long term breeding program of hybrid larch (*Larix* × *eurolepis* Henry). *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 75-80. (Queensland Department of Primary Industries, Brisbane).

Schonau, A.P.G. (Eds.) (1991). 'Intensive Forestry: The Role of Eucalypts.' *Proc. of* IUFRO symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Southern African Institute of Forestry, Pretoria).

Sedgley, M. and Griffin, A.R. (1989). 'Sexual reproduction of tree crops'. (Academic Press, London).

Sedgley, M., Hand, F.C., Smith, R.M. and Griffin, A.R. (1989). Pollen tube growth and early seed development in *Eucalyptus regnans* F. Muell. (Myrtaceae) in relation to ovule structure and preferential outcrossing. *Australian Journal of Botany* 37, 397-411.

Sedgley, M. and Smith, R.M. (1989). Pistil receptivity and pollen tube growth in relation to the breeding system of *Eucalyptus woodwardii* (*Symphyomyrtus: Myrtaceae*). *Annals of Botany* 64, 21-32.

Shelbourne, C.J.A. (1991). Genetic gains from different kinds of breeding populations and seed or plant production population. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schönau) pp. 300-317. (Southern African Institute of Forestry, Pretoria).

Shelbourne, C.J.A. (2000). Some insights on hybrids in forest tree improvement. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 53-62. (Queensland Department of Primary Industries, Brisbane).

Shelbourne, T., Apiolaza, L.A., Jayawickrama, K.J.S. and Sorensson, C.T. (1997). Developing breeding objectives for radiata pine in New Zealand. *In* 'Genetics of Radiata Pine'. *Proc. of* IUFRO Conference. Rotorua, New Zealand. (Eds. R.D. Burdon and J.M. Moore) pp. 160-168. (New Zealand Forest Research Institute Ltd (FRI Bulletin No.203).

Soria, F. and Borralho, N.M.G. (1998). The genetics of resistance to *Phoracantha semipunctata* attack in *Eucalyptus globulus* in Spain. *Silvae Genetica* 46, 365-369.

Squillace, A.E. (1974). Average genetic correlations among offspring from open-pollinated forest trees. *Silvae Genetica* 23, 149-156.

Stonecypher, R.W. and McCullough, R.B. (1981). Estimates of additive and non additive genetic variances from a clonal diallel of Douglas Fir (*Pseudotsuga menziesii* (Mirb.) Franco.). *Proc. of* 16th Southern Forest Tree Improvement Conference pp. 2-17. (US Forest Service, Athens, Georgia).

Talbert, C.B. (1995). From gene to market: Realizing the value from genetics and silviculture as an integrated system. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 20-23. (CRC for Temperate Hardwood Forestry, Hobart).

Templeton, A.R., Hemmer, H., Mace, G., Seal, U.S., Shields, W.M. and Woodruff, D.S. (1986). Local adaptation, coadaptation, and population boundaries. *Zoo Biology* 5, 115-125.

Tibbits, W.N. (1986). Eucalypt plantations in Tasmania. *Australian Forestry* 49, 219-225.

Tibbits, W.N. (1989). Controlled pollination studies with shining gum (*Eucalyptus nitens* (Deane and Maiden) Maiden). *Forestry* 62, 111-126.

Tibbits, W.N. (1991). Flowering and seed yields in orchards of *Eucalyptus nitens* (D&M) Maiden. *In Proc. of* 11th Meeting of the Research Working Group 1 (Forest Genetics) of the Australian Forestry Council. Coonawarra, South Australia, Mar. 1991. (Ed. C.A. Dean) p. 79. (CSIRO, Mt. Gambier).

Tibbits, W.N. (2000). Evaluation of hybrids with *Eucalyptus nitens*. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* 'QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 363-372. (Queensland Department of Primary Industries, Brisbane).

Tibbits, W.N., Boomsma, D.B. and Jarvis, S. (1997a). Distribution, biology, genetics, and improvement programs for *Eucalyptus globulus* and *E. nitens* around the world. *In Proc. of* 24th Biennial Southern Forest Tree Improvement Conference. Orlando, Florida, USA, 9-12 Jun. 1997. (Eds. T. White, D. Huber and G. Powell) pp. 81-95. (Southern Tree Improvement Committee and University of Florida, Gainesville).

Tibbits, W.N., de Little, D.W. and Ravenwood, I.C. (1989). Improving short-rotation, fast-growing, cool temperate eucalypts through controlled pollination, hybridisation and vegetative propagation. *In Proc. of* Thirteenth Commonwealth Forestry Conference. Rotorua, New Zealand, 17-30 Sep. 1989 pp. 319-324. (Ministry of Forestry, Wellington, NZ).

Tibbits, W.N., Dean, G.H. and French, J. (1995). Relative pulping properties of *Eucalyptus nitens* x *E. globulus* F1 hybrids. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 83-84. (CRC for Temperate Hardwood Forestry, Hobart).

Tibbits, W.N. and Hodge, G.R. (1995). Making second generation *Eucalyptus nitens* selections for wood fibre production. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 267-270. (CRC for Temperate Hardwood Forestry, Hobart).

Tibbits, W.N. and Hodge, G.R. (1998). Genetic parameters and breeding value predictions for *Eucalyptus nitens* wood fiber production traits. *Forest Science* 44, 587-598.

Tibbits, W.N. and Hodge, G.R. (2001). 'Genetic parameters for cold hardiness in *Eucalyptus nitens* (Deane & Maiden) Maiden.' CRCSPF Technical Report No. 49. (CRC Sustainable Production Forestry, Hobart).

Tibbits, W.N., Hodge, G.R. and White, T.L. (1991a). Predicting breeding values for freezing resistance in *Eucalyptus globulus*. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schonau) pp. 330-333. (Southern African Institute of Forestry, Pretoria).

Tibbits, W.N., Potts, B.M. and Savva, M.H. (1991b). Inheritance of freezing resistance in interspecific F_1 hybrids of *Eucalyptus*. *Theoretical and Applied Genetics* 83, 126-135.

Tibbits, W.N. and Reid, J.B. (1987a). Frost resistance in *Eucalyptus nitens* (Deane & Maiden) Maiden: Genetic and seasonal aspects of variation. *Australian Forest Research* 17, 29-47.

Tibbits, W.N. and Reid, J.B. (1987b). Frost resistance in *Eucalyptus nitens* (Deane & Maiden) Maiden: Physiological aspects of hardiness. *Australian Journal of Botany* 35, 235-250.

Tibbits, W.N., White, T.L. and Hodge, G.R. (1997b). Genetic control of rooting ability of stem cuttings in *Eucalyptus nitens*. *Australian Journal of Botany* 45, 203-210.

Turnbull, J.W. and Pryor, L.D. (1984). Choice of species and seed sources. *In* 'Eucalypts for Wood Production'. (Eds. W.E. Hillis and A.G. Brown) pp. 6-176. (CSIRO;Academic Press, Melbourne;Sydney).

van Wyk, G. (1976). Early growth results in a diallel progeny test of *Eucalyptus grandis* (Hill) Maiden. 1. A field study. *Silvae Genetica* 25, 126-132.

van Wyk, G. (1977). Early growth results in a diallel progeny test of *Eucalyptus grandis* (Hill) Maiden. II. A greenhouse study. *Silvae Genetica* 26, 44-50.

van Wyk, G. (1985). Tree breeding in support of vegetative propagation of *Eucalyptus grandis* (Hill) Maiden. *South African Forestry Journal* 135, 33-39.

van Wyk, G. (1990). Genetic improvement of timber yield and wood quality in *Eucalyptus grandis* (Hill) Maiden. Part 1. Genetic parameters of growth characteristics. *South African Forestry Journal* 153, 1-11.

Venkatesh, C.S. (1982). A strategy for accelerated selection and release of advanced-generation eucalypt species hybrids. *Indian Forester* 108, 247-252.

Venkatesh, C.S. and Sharma, V.K. (1976). Heterosis in the flowering precocity of *Eucalyptus* hybrids. *Silvae Genetica* 25, 28-29.

Venkatesh, C.S. and Sharma, V.K. (1977a). Hybrid vigour in controlled interspecific crosses of *Eucalyptus tereticornis* x *E. camaldulensis*. *Silvae Genetica* 26, 121-124.

Venkatesh, C.S. and Sharma, V.K. (1977b). Rapid growth rate and higher yield potential of heterotic *Eucalyptus* species hybrids. *Indian Forester* 103, 795-802.

Venkatesh, C.S. and Sharma, V.K. (1979). Comparison of a *Eucalyptus tereticornis* X *E. grandis* controlled hybrid with a *E. grandis* X *E. tereticornis* putative natural hybrid. *Silvae Genetica* 28, 127-131.

Venkatesh, C.S. and Thapliyal, R.C. (1993). Heterosis and variation in reproductive parameters of reciprocal F_1 hybrids between *Eucalyptus tereticornis* Sm. and *E. camaldulensis* Dehn. *Indian Forester* 119, 714-721.

Verryn, S.D. (2000). Eucalypt hybrid breeding in South Africa. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 191-199. (Queensland Department of Primary Industries, Brisbane).

Vigneron, P. (1988). Les hybrides artificiels d'*Eucalyptus* au Congo. Création et multiplication. *In* 'Population structure and genetic improvement strategies in clonal and seedling forestry'. *Proc. of* IUFRO Conference on Breeding Tropical Trees. Pattaya, Thailand, 28 November-3 December 1988. (Eds. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 425-427. (Oxford Forestry Institute, Oxford, United Kingdom, Winrock International, Arlington, Virginia USA).

Vigneron, P. (1991). Creation et amelioration de varietes hybrids d'eucalyptus au Congo. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schönau) pp. 345-360. (Southern African Institute of Forestry, Pretoria).

Vigneron, P. (1995). Production and improvment of *Eucalyptus* varietal hybrids in the Congo. *In* 'Traitements statistiques des essais de selection: strategies d'amelioration des plantes perennes'. *Proc. of* Actes du seminaire de biometrie et genetique quantitative. Montpellier, France, 12-14 Sep. 1995 pp. 259-273. (CIRAD, Montpellier, France).

Vigneron, P. and Bouvet, J.M. (2000). Eucalypt hybrid breeding in Congo. *In* 'Hybrid Breeding and Genetics of Forest Trees'. *Proc. of* QFRI/CRC-SPF Symposium. Noosa, Queensland, Australia, 9-14 Apr. 2000. (Eds. H.S. Dungey, M.J. Dieters and D.G. Nikles) pp. 14-26. (Queensland Department of Primary Industries, Brisbane).

Volker, P. and Raymond, C.A. (1989). Potential for breeding eucalypts in Tasmania. *Appita* 42, 198-199.

Volker, P.W. (1995). Evaluation of *Eucalyptus nitens* x *globulus* for commercial forestry. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 222-225. (CRC for Temperate Hardwood Forestry, Hobart).

Volker, P.W., Dean, C.A., Tibbits, W.N. and Ravenwood, I.C. (1990). Genetic parameters and gains expected from selection in *Eucalyptus globulus* in Tasmania. *Silvae Genetica* 39, 18-21.

Volker, P.W. and Orme, R.K. (1988). Provenance trials of *Eucalyptus globulus* and related species in Tasmania. *Australian Forestry* 51, 257-265.

Volker, P.W., Owen, J.V. and Borralho, N.M.G. (1994). Genetic variances and covariances for frost tolerance in *Eucalyptus globulus* and *E. nitens*. *Silvae Genetica* 43, 366-372.

Wei, X.M. and Borralho, M.G. (2000). Genetic gains and levels of relatedness from best linear unbiased prediction selection of Eucalyptus urophylla for pulp production in southeastern China. *Canadian Journal of Forest Research* 30, 1601-1607.

White, T.L. (1987). A conceptual framework for tree improvement programs. *New Forests* 4, 325-342.

White, T.L. and Hodge, G.R. (1989). 'Predicting Breeding Values With Applications in Forest Tree Improvement.'. (Kluwer Academic Publishers, London, Dordrecht).

Whiteman, P.H., Dean, C.A., Doran, J.C. and Cameron, J.N. (1992). Genetic parameters and selection strategies for *Eucalyptus nitens* (Dean and Maiden) in Victoria. *Silvae Genetica* 41, 77-81.

Wilcox, M.D. (1982). Genetic variation in frost tolerance, early height growth, and incidence of forking among and within provenances of *Eucalyptus fastigata*. *New Zealand Journal of Forestry Science* 12, 510-524.

Wilcox, M.D., Faulds, T., Vincent, T.G. and Poole, B.R. (1980). Genetic variation in frost tolerance among open-pollinated families of *Eucalyptus regnans* F. Muell. *Australian Forest Research* 10, 169-184.

Williams, D.R., Potts, B.M. and Black, P.G. (1999). A single visit pollination method for *Eucalyptus globulus*. *Australian Forestry* 62, 346-352.

Williams, M.D., Beadle, C.L., Turnbull, C.R., Dean, G.H. and French, J. (1995). Papermaking potential of plantation eucalypts. *In* 'Eucalypt Plantations: Improving Fibre Yield and Quality'. *Proc. of* CRCTHF-IUFRO Conf. Hobart, Tasmania, Australia, 19-24 Feb. 1995. (Eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond) pp. 73-78. (CRC for Temperate Hardwood Forestry, Hobart).

Willing, R.R. and Pryor, L.D. (1976). Interspecific Hybridisation in Poplar. *Theoretical and Applied Genetics* 47, 141-151.

Wilson, P.J. (1993). Propagation charateristics of *Eucalyptus globulus* Labill. spp. *globulus* stem cuttings in relation to their original position in the parent shoot. *Journal of Horticultural Science.* 68, 715-724.

Wilson, P.J. (1995). Getting back to the roots of tree improvement. *Pulp and Paper International* 37, 42-43.

Wilson, P.J. (1996). Multiplication rates in vitro and by stem cuttings propagation, and clonal development from *Eucalyptus globulus* seedlings. *Forest Science* 42, 415-418.

Wilson, P.J. (1998). Developing clones from *Eucalyptus globulus* and hybrid seedlings by stem cuttings propagation. *New Zealand Journal of Forestry Science* 28, 293-303.

Wilson, P.J. (1999). Developing clones from field-grown coppice of *Eucalyptus globulus* by stem cuttings propagation. *Southern African Forestry Journal* 185, 45-49.

Woolaston, R., Whiteman, P.H. and Cameron, J.N. (1991a). Genetic analyses of four year diameter measures for *Eucalyptus globulus* growing across four sites in Victoria, Australia. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schönau) pp. 371-381. (Southern African Institute of Forestry, Pretoria).

Woolaston, R., Whiteman, P.H. and Cameron, J.N. (1991b). Genetic analyses of four year diameter measures for *Eucalyptus nitens* growing across three sites in Victoria, Australia. *In* 'Intensive Forestry: The Role of Eucalypts'. *Proc. of* IUFRO Symposium, P2.02-01 Productivity of Eucalypts. Durban, South Africa, 2-6 Sep. 1991. (Ed. A.P.G. Schönau) pp. 361-370. (Southern African Institute of Forestry, Pretoria).

Wright, J.A. (1997). A review of the worldwide activities in tree improvement for *Eucalyptus grandis, Eucalyptus urophylla* and the hybrid *urograndis*. *In Proc. of* 24th Biennial Southern Forest Tree Improvement Conference. Orlando, Florida, USA, 9-12 Jun. 1997. (Eds. T. White, D. Huber and G. Powell) pp. 96-102. (Southern Forest Tree Improvement Committee and University of Florida, Gainesville).

Wright, J.W. (1964). Hybridization between species and races. *Unasylva* 18, 30-39.

Wright, J.W. (1976). 'Introduction to Forest Genetics'. (Academic Press, New York).

Zobel, B.J. (1993). Clonal forestry in the eucalypts. *In* 'Clonal Forestry II. - Conservation and Application'. (Eds. M.R. Ahuja and W.J. Libby) pp. 139-148. (Springer-Verlag, Berlin).

Zobel, B.J. and Jett, J.B. (1995). 'Genetics of Wood Production'. (Springer-Verlag, Berlin).

Zobel, B.J. and Talbert, J.T. (1984). 'Applied Forest Tree Improvement'. (John Wiley & Sons, New York).